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(54) Title: COMPOSITIONS OF AN AQUAPORIN MODULATING AGENT AND AN AQUEOUS HUMOR MODULATING AGENT FOR THE TREATMENT OF ELEVATED INTRAOCULAR PRESSURE

(57) Abstract: The present invention provides compositions and methods for lowering intraocular pressure in a subject. More particularly, the invention provides a combination therapy for the treatment of an ophthalmic disorder mediated by an elevated intraocular pressure comprising administering to a subject an aquaporin modulating agent in combination with an aqueous humor modulating agent, where the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin.

COMPOSITIONS OF AN AQUAPORIN MODULATING AGENT AND AN AQUEOUS HUMOR MODULATING AGENT FOR THE TREATMENT OF ELEVATED INTRAOCULAR PRESSURE

Field of the Invention

[0001] The present invention provides compositions and methods for lowering intraocular pressure. More particularly, the invention is directed toward a combination therapy for the treatment of an ophthalmic disorder mediated by elevated intraocular pressure comprising administering to a subject an aquaporin modulating agent in combination with an aqueous humor modulating agent.

Background of the Invention

[0002] The continued increase in the incidence of ophthalmic disorders mediated by elevated intraocular pressure (IOP), including glaucoma, provides compelling evidence that there is a continuing need for better treatment strategies. Glaucoma, for example, is consistently among the leading causes of blindness and optic nerve damage among adults in the United States. Generally speaking, glaucoma is characterized by a progressive neuropathy caused in part by deleterious effects resulting from increased IOP on the optic nerve. In normal individuals, IOPs range from 12 to 20 mm Hg., averaging approximately 16 mm Hg. But in individuals suffering from glaucoma, IOPs typically rise to 25 mm Hg. or greater, and can sometimes exceed 40 mm Hg resulting in rapid and permanent visual loss. Loss of vision can result from IOPs only slightly above the normal range in eyes that are unusually pressure-sensitive over a period of years. Moreover, extremely high pressures, e.g., 70 mm Hg., may cause blindness within only a few days if left untreated.

[0003] Two mainstays of glaucoma treatment are decreasing aqueous humor production, or enhancing its outflow from the eye. Aqueous humor is the fluid that fills the chamber of the eye behind the cornea and in front of the lens. It is formed through the ciliary body, and is secreted constantly into the posterior chamber resulting in a continual flow between the iris and the lens and through the pupil into the chamber of the eye. In individuals with an IOP in the normal range, aqueous humor concentration is maintained as a delicate equilibrium mediated by the balance between its production and outflow. When everything functions

correctly, ocular pressure is normal and aqueous humor inflow is approximately equal to outflow. But when this equilibrium is disrupted by factors such as aging, inflammation, hemorrhage, or cataracts, IOP may become dangerously elevated if left untreated.

[0004] All therapies currently employed to treat ophthalmic disorders mediated by elevated IOP are restricted to reducing IOP by either affecting the production or outflow of aqueous humor. Depending upon the type and severity of the condition, either surgical or pharmacological treatments may be employed to lower IOP. By way of example, both laser and incisional surgical procedures may be used for the treatment of severe conditions such as open-angle glaucoma. Angle-closure glaucoma entails closure or blockage of the anterior chamber angle, thereby restricting outflow of aqueous humor. While pharmacological agents generally effectively control mild cases of open-angle glaucoma, laser trabeculoplasty or filtering surgery to improve aqueous drainage is employed in severe cases. Though often necessary and quite effective for many types of glaucoma, surgical intervention is an invasive form of treatment, even if local anesthesia can be used.

[0005] Several classes of pharmacological agents may also be employed to lower IOP. One such class of pharmacological agent is miotic agents. Though their precise mechanism of action has not yet been fully elucidated, miotic drugs lower IOP by facilitating aqueous humor outflow. Mydriatic agents are also useful for lowering IOP. For example, the sympathomimetic amines, such as epinephrine and dipivefrin, lower IOP, at least in part through stimulation of $beta_2$ -adrenergic receptors in the trabecular meshwork. Additionally, $alpha_2$-adrenergic agonists (e.g. apraclonidine) have been shown to be effective in lowering IOP by inhibition of aqueous humor formation. Moreover, both non-selective $beta_1$- and $beta_2$ -adrenergic blocking agents (e.g., timolol and levobunolol) and $beta_1$-selective (e.g., betaxolol) adrenergic blocking agents are also used to lower IOP. Prostaglandin compounds have also been shown to have an ocular hypotensive activity. Although these pharmacological agents are all less invasive than surgical intervention, they never-the-less are still often accompanied by adverse effects (e.g. conjunctival irritation, burred vision, ocular pain, and headaches) at the dosages required for effective treatment.

[0006] Aquaporins (AQP), a large family of membrane proteins that function as highly selective water channels, have also been identified as a target for

modulating IOP. At least ten AQPs, numbered 0 through 9, have been identified from various mammalian tissues (e.g. brain, kidney, salivary gland, testis, and liver) and AQPs 0 through 5 have been identified in the eye. Several studies have described functional roles for AQPs in ocular physiology. For example, inhibition of AQP1 using antisense oligonucleotides reduces the fluid movement across the ciliary epithelial cells in culture (Hamann et al., (1998) Am. J. Physiol. 274:C1332-1345); and mutations in AQP0 result in congenital cataracts (Shiels, A. and Bassnett, S. (1996) Nature Genet 12:212-215). It was also shown that AQP1-knockout mice have lower IOP and aqueous humor production (Zhang et al., (2002) J. Gen Physiol 119:561-569).

Summary of the Invention

[0007] Among the aspects of the present invention is provided a method for lowering IOP in a subject comprising administering to the subject an aquaporin modulating agent in combination with an aqueous humor modulating agent, where the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin.

[0008] Another aspect of the invention provides a method to treat an ophthalmic disorder mediated by an elevated IOP in a subject comprising administering to the subject an aquaporin modulating agent and an aqueous humor modulating agent, where the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin. In one embodiment, the ophthalmic disorder is a glaucoma disorder. In one alternative of this embodiment, the glaucoma disorder is primary angle closure glaucoma. In another alternative of this embodiment, the glaucoma disorder is secondary open angle glaucoma. In another embodiment, the ophthalmic disorder is ocular hypertension.

[0009] In still another aspect of the invention is provided a method to treat a glaucoma disorder in a subject comprising administering to the subject an aquaporin modulating agent and an aqueous humor modulating agent. In one embodiment, the glaucoma disorder is primary angle closure glaucoma. In another embodiment, the glaucoma disorder is secondary open angle glaucoma.

[0010] A further aspect of the invention provides a composition comprising an aquaporin modulating agent and an aqueous humor modulating agent.

[0011] In one embodiment, the aquaporin modulating agent alters the expression of aquaporin. In another embodiment, the agent alters expression by substantially inhibiting aquaporin gene expression. In one alternative of this embodiment, the aquaporin expression inhibitor is a carbonic anhydrase inhibitor, vasopressin, or an angiotensin converting enzyme inhibitor. In another alternative of this embodiment, the aquaporin expression inhibitor is an aquaporin antisense oligonucleotide or a ribozyme.

[0012] In another embodiment, the aquaporin modulating agent inhibits or enhances the function of aquaporin. In one alternative of this embodiment, the aquaporin modulating agent is a protein kinase C activator. In another alternative of this embodiment, the aquaporin modulating agent is a protein kinase A inhibitor.

[0013] In yet another embodiment, the aqueous humor modulating agent is a prostaglandin, a beta adrenergic antagonist blocker, an adrenergic agonist, a cholinergic agonist, or a carbonic anhydrase inhibitor.

[0014] Other aspects and features of the invention are described in more detail below.

Definitions and Abbreviations

[0015] The term "subject" for purposes of treatment includes any human or animal subject who is susceptible to an elevated IOP. The subject can be a domestic livestock species, a laboratory animal species, a zoo animal or a companion animal. In one embodiment, the subject is a mammal. In another embodiment, the mammal is a human being.

[0016] The phrase "therapeutically-effective" is intended to qualify the amount of each agent (i.e. the amount of AQP modulating agent and the amount of aqueous humor modulating agent) that will achieve the goal of improvement in disorder severity and the frequency of incidence over no treatment or treatment of each agent by itself.

Description of the Preferred Embodiments

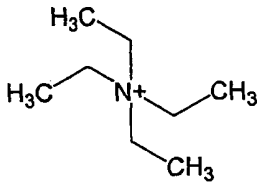
[0017] The present invention provides a combination therapy comprising the administration to a subject of a therapeutically effective amount of an AQP modulating agent in combination with a therapeutically effective amount of an aqueous humor modulating agent, where the aqueous humor modulating agent

lowers intraocular pressure by a pathway other than the modulation of aquaporin. The combination therapy is used to lower IOP, and to treat ophthalmic disorders mediated by elevated IOP. When administered as part of a combination therapy, the AQP modulating agent together with the aqueous humor modulating agent provide enhanced treatment options as compared to administration of either the AQP modulating agent or the aqueous humor modulating agent alone.

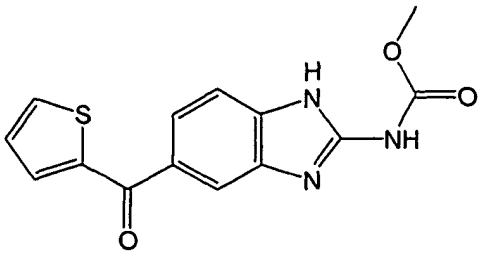
Aquaporin Modulating Agents

[0018] In general, one aspect of the present invention is the use of an AQP modulating agent that lowers IOP. Typically, the agent selected will lower IOP by reducing the production of aqueous humor via either modulating the expression of AQP or by modulating its function once expressed. The agent may also lower IOP by modifying the secretion of aqueous humor from the eye once it is produced. By way of example, the agent selected may lower IOP by increasing the outflow of aqueous humor from the anterior chamber of the eye. Moreover, the agent selected may be effective in modulating any of the various AQP isoforms, including AQP0 through AQP9, to the extent that modulating the isoform lowers IOP. Because of their prevalence in the eye, however, typically the agent will modulate one or more of AQP0 through AQP5 and more typically, the agent will modulate AQP1 or AQP4.

[0019] In one embodiment, the AQP modulating agent is tetraethylammonium or a pharmaceutically acceptable salt having the structure:



[0020] In another embodiment, the AQP modulating agent is any such agent described in WO 01/64219 A2, which is hereby incorporated by reference in its entirety. In one alternative of this embodiment, the AQP modulating agent is nocodazole or a pharmaceutically acceptable salt having the structure:



[0021] In still another alternative of this embodiment, the AQP modulating agent is a vinca alkyloid. For example, suitable vinca alkyloids include vincristine, vinblasine, and vinorelbine. In a further alternative of this embodiment, the AQP modulating agent is selected from the group consisting of colchicine, rhizoxin, estramustine, erbuluzole, tubulozole, and cytochalasin D.

[0022] Another aspect of the invention encompasses AQP modulating agents that lower IOP by altering the expression of an AQP gene. In some aspects, the agent may cause a decrease in the overall rate of AQP gene expression and concomitantly, result in a decrease in mature AQP. In other aspects, the agent may cause an increase in the overall rate of AQP gene expression. Likewise, the agent may modify expression of an AQP gene such that the amount of functional AQP decreases or increases. By way of example, the agent may cause premature termination of AQP gene transcription, thereby resulting in a shorter transcription product. By way of further example, the agent may alter or interrupt the sequence of the transcription product such that proper post transcription processing and translation of a functional AQP does not occur or occurs at a substantially reduced rate.

[0023] In one embodiment, the AQP modulating agent is an AQP antisense oligonucleotide. These agents are typically unmodified or modified antisense oligonucleotides directed against various AQP nucleic acid sequences that inhibit AQP gene transcription in both a sequence-specific and in a non-sequence specific manner. Because of their complementary, the agent binds to the AQP nucleic acid and thereby prevents its transcription. Of course, the particular antisense oligonucleotides employed will vary considerably depending upon its intended target within the AQP gene and one skilled in the art can readily design appropriate antisense oligonucleotides for use in the present invention. Methods for selecting and constructing antisense oligonucleotides suitable for use in the

invention are more fully described, for example, in Hamann et al., (1998) Am. J. Physiol. 274:C1332-1345.

[0024] In yet another embodiment, the AQP modulating agent is a ribozyme. Ribozymes are RNA molecules having an enzymatic activity that are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Within the context of the present invention, the ribozyme employed typically cleaves AQP expressed RNA and in particular, mRNA targets, resulting in the destruction of mRNA transcript integrity. By way of example, the ribozyme employed may be targeted to and prevents the translation of mRNA encoding a region of AQP required for proper translation or translocation. By way of further example, the ribozyme employed may be targeted to and prevents the translation of mRNA encoding a region of AQP required for proper function of the mature protein.

[0025] In still another embodiment, the AQP modulating agent is a carbonic anhydrase (CA) inhibitor. A number of different CA inhibitors capable of lowering IOP by altering the expression of an AQP gene may be employed. Generally speaking, the CA inhibitor may inhibit any isomer of the metalloprotein enzyme that catalyzes the interconversion of CO_2 and H_2CO_3 ($CO_2 + O_2 \rightarrow HCO_2^- + H^+$). Typically, however, the CA inhibitor will inhibit either the CAII or CAIV isoform. By way of example, the CA inhibitor acetazolamide results in a significant decrease in the level of AQP1 expression in the epididymis of rats (Yu et al., (2002) Arch Androl 48(4):281-294). Other suitable CA inhibitors include methazolamide, dorzolamide hydrochloride ophthalmic solution, dorzolamide hydrochloride-timolol maleate ophthalmic solution, brinzolamide hydrochloride, dorzolamide, and brinzolamide.

[0026] In a further embodiment, the AQP modulating agent is an angiotensin converting enzyme inhibitor. A number of angiotensin converting enzyme inhibitors capable of lowering IOP by altering the expression of an AQP gene may be utilized. By way of example, angiotensin II increases the expression of AQP2 in the kidney of cardiomyopathic hamsters (Wong NL, and Tsui JK, (2002) Metabolism 51(8):970-975) . Administration of the angiotensin converting enzyme inhibitor enalapril to the cardiomyopathic hamsters causes a significant decrease in the level of AQP2 expression so that it is comparable to the level of AQP2 expressed in normal hamsters (i.e. hamster that are not cardiomyopathic). Other angiotensin converting enzyme inhibitors suitable for use in the present invention include

benazepril, captopril, fosinopril, lisinopril, moexipril, quinapril, ramipril, and trandolapril.

[0027] Yet another aspect of the invention encompasses AQP modulating agents that substantially alter the function of AQP. In some aspects, the agent may disrupt the ability of AQP to form a fluid membrane channel. For example, the agent may prevent proper assembly of AQP subunits such that AQP cannot embed within the plasma membrane and form a channel. Likewise, the agent may disrupt the ability of AQP to function as a fluid membrane channel. By way of example, the agent may bind to an AQP of a functional membrane channel and either permanently or transiently prevent the ability of fluid to pass through the channel. In other aspects, the agent may prevent the ability of AQP to form a gated ion channel, such as a cyclic GMP gated ion channel. By way of example, the agent may prevent phosphorylation of AQP at a site necessary for its ability to function as a gated ion channel. By way of further example, the agent may inactivate an intermediary compound necessary for AQP function.

[0028] In one embodiment, the AQP modulating agent is a protein kinase C (PKC) activator. PKC is a member of the protein kinase family responsible for regulating pathways of intermediary metabolism (e.g. glycogen phosphorylase kinase). Typically, when a PKC activating agent is employed, the AQP target is generally AQP4 (see e.g. Han et al., (1998) J. Biol. Chem. 273:6001-6004, demonstrating that the water channel activity of AQP4 is in part regulated by protein phosphorylation via a PKC pathway). Within the context of the invention, a number of agents that result in the activation of PKC may be employed. In one regulatory pathway, activation of PKC occurs when plasma membrane receptors coupled to phospholipase C are themselves activated causing the release of diacylglycerol, which in turn activates PKC. Generally speaking, the agent will typically be a diacylglycerol mimic that can directly activate PKC. In one aspect of this embodiment, the diacylglycerol mimic is a phorbol ester. Phorbol esters suitable for use in the present invention include phorbol 12, 13 dibutyrate, phorbol 12-myristate-12-acetate, phorbol 12-O-tetradecanoylphorbol 13-acetate, phorbol 12, 13 didecanoate and tetradecanoylphorbol acetate. In other aspects of this embodiment, the agent employed may indirectly activate PKC by activating phospholipase C causing the release of diacylglycerol. Likewise, the agent may activate PKC by a pathway that is independent from the diacylglycerol pathway. By way of example,

ionomycin is a molecule that carries calcium through the plasma membrane to increase the calcium concentration in the cytoplasm and activate PKC without activating phospholipase C.

[0029] In another embodiment, the AQP modulating agent is an adenylate cyclase inhibitor. Adenylate cyclase is a membrane bound enzyme that converts adenosine triphosphate (ATP) to 3', 5'-cyclic adenosine monophosphate (cAMP), which is a potent intracellular messenger. Accordingly, inhibition of adenylate cyclase concomitantly causes a reduction in intracellular cAMP levels. Typically, when an adenylate cyclase inhibitor is utilized, the AQP target is generally AQP1 (see *e.g.* Patil et al., (1997) Science 275:1492, demonstrating that the water channel activity of AQP1 is in part regulated by atrial natriuetic peptide, a known adenylate cyclase inhibitor). Within the context of the invention, a number of agents that result in the inhibition of adenylate cyclase may be employed. In one aspect of this embodiment, the agent is a natriuretic peptide that inhibits adenylate cyclase. Natriuretic peptides are any of several proteins that stimulate natriuresis. By way of example, suitable natriuretic peptides for use in the present invention include atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).

[0030] In yet another embodiment, the AQP modulating agent inhibits a cAMP dependent protein kinase such as protein kinase A (PKA). PKA belongs to a class of protein kinases that are regulated by cAMP. Typically, when a PKA inhibitor is employed, the AQP target is generally AQP1 (see e.g. Yoo et al., (1996) Science 273(5279) 1216-1218, demonstrating that the water channel activity of AQP1 is in part regulated by a cAMP dependent mechanism via a PKA pathway). Within the context of the invention, a number of agents that result in the inhibition of PKA may be employed. Examples of suitable PKA inhibitors include (5-isoquinolinesulfonyl)piperazine; 1-(5-Isoquinolinesulfonyl)-2-methylpiperazine, 4-cyano-3-methylisoquinoline; adenosine 3',5'-cyclic monophosphorothioate, 2'-O-monobutyryl; adenosine 3',5'-cyclic monophosphorothioate; 8-bromo-2'-monobutyryl, adenosine 3',5'-cyclic monophosphorothioate; 8-piperidino, N-(2-aminoethyl)-5-chloronaphthalene-1-sulfonamide; N-(2-aminoethyl)-5-isoquinolinesulfonamide; N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide; N-(2-guanidinoethyl)-5-isoquinolinesulfonamide; 4,4',5,5',6,6'-hexahydroxydiphenic acid 2,6,2',6'-dilactone; (5-isoquinolinesulfonyl) homopiperazine; N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide; and *trans*-3,3',4,5'-tetrahydroxystilbene.

[0031] In still a further embodiment, the AQP modulating agent is a vasoactive peptide. As a class, vasoactive peptides are typically peptides that affect the diameter of a blood vessel. Typically, when a vasoactive peptide is utilized, the AQP target is generally AQP1 (see e.g. Patil et al., (1997) Biochem. Biophys. Res. Comm. 238:392-396, demonstrating that the water channel activity of AQP1 is in part regulated by the vasoactive peptides atrial natriuetic peptide and arginine vasopressin). Within the context of the invention, a number of vasoactive peptides that result in an inhibition of any AQP function may be employed. In one alternative of this embodiment, the vasoactive peptide is a vassopressin, such as arginine vasopressin. In another aspect of the invention, the vasoactive peptide is a natriuretic peptide such as ANP or BNP.

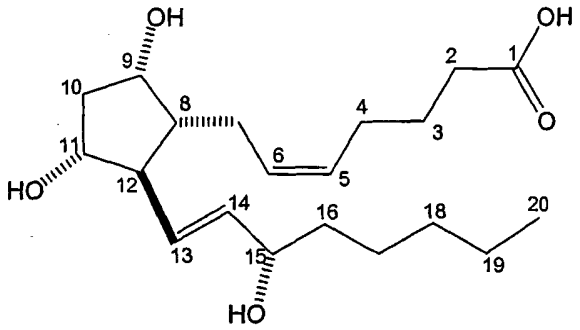
[0032] Of course it will be apparent to a skilled artisan that a particular AQP modulating agent may modulate AQP by a number of different mechanism. For example, a specific AQP modulating agent may decrease the expression of AQP and substantially inhibit its function once expressed. In other aspects, the AQP modulating agent may not impact either the expression or function of AQP. It is contemplated that all AQP modulating agents that lower IOP are within the scope of the invention irrespective of their mechanism of action.

Aqueous Humor Modulating Agents

[0033] In addition to an AQP modulating agent, the composition also an aqueous humor modulating agent. A number of aqueous humor modulating agents may be employed to the extent that they lower IOP. In general, the aqueous humor modulating agent may lower IOP by causing a reduction in the formation of aqueous humor. The aqueous humor modulating agent may also lower IOP by increasing the outflow of aqueous humor from the anterior chamber of the eye. Moreover, the aqueous humor modulating agent may lower IOP by decreasing the inflow of aqueous humor from the anterior chamber of the eye. Irrespective of a particular aqueous humor modulating agent's mechanism of action, it typically lowers IOP by a pathway other than the modulation of AQP.

[0034] In one aspect, the aqueous humor modulating agent is a prostaglandin or a prostaglandin analog. Naturally occurring prostaglandins are C-20 unsaturated fatty acids. Typically, any prostagladin or prostaglandin analog capable of lowering IOP by altering the production, inflow or outflow of aqueous

humor may be used in the composition. Suitable prostaglandins that may be employed in the composition include prostaglandin A, prostaglandin B, prostaglandin D, prostaglandin E, prostaglandin F or any combination thereof. Typically, the prostaglandin employed is prostaglandin F or a homolog of prostaglandin F such as PGF_{2a}. By way of example, PGF_{2a} is characterized by hydroxyl groups at the C_9 and C_{11} positions on the alicyclic ring, a cis-double bond between C_5 and C_6, and a trans-double bond between C_{13} and C_{14}. PGF_{2a} has the following formula:



[0035] In another embodiment, the aqueous humor modulating agent is a prostaglandin analog. Typically, suitable prostaglandin analogs include any analogs that are similar in structure and function to prostaglandin, which lower IOP. In one alternative of this embodiment, the prostaglandin analog is a prostaglandin FP receptor antagonist. In another alternative of this embodiment, the prostaglandin analog is a prostaglandin F_{2a} analog. In one embodiment, the prostaglandin F_{2a} analog is lanaprost. In another embodiment, the F_{2a} analog is travoprost. In still a further alternative of this embodiment, the prostaglandin analog is unoprostone. In a further alternative of this embodiment, the prostaglandin analog is a prostamide. Generally speaking, the prostamide employed may be any naturally occurring or synthetic prostamide. In one embodiment, the prostamide is the synthetic analog bimatoprost. The preparation and pharmaceutical profiles of several prostaglandin and prostaglandin analogs, including cloprostenol, fluprostenol, latanoprost, and travoprost, are more fully described in U.S. Patent No. 5,510,383, which is hereby incorporated by reference in its entirety.

[0036] In a further aspect, the aqueous humor modulating agent is a beta adrenergic receptor antagonists. Beta adrenergic receptor antagonists bind beta-adrenergic receptors such as the $beta_1$ adrenergic receptor or the $beta_2$ adrenergic receptor. By binding to these receptors, the beta adrenergic receptor antagonists decrease the ability of the body's own natural epinephrine to bind to those receptors,

leading to inhibition of various processes in the body's sympathetic system, including a reduction in aqueous humor secretion by ciliary tissues in the eye. Generally speaking, any beta adrenergic receptor antagonists capable of lowering IOP by altering the production, inflow or outflow of aqueous humor may be used in the composition. In some embodiments, the beta adrenergic receptor antagonists may be selective for the $beta_1$ adrenergic receptor. By way of example, suitable selective $beta_1$ adrenergic receptor antagonists include betaxolol and its enantiomer levobetaxolol. In other embodiments, the beta adrenergic receptor antagonists may be non-selective, blocking both the $beta_1$ adrenergic receptor and the $beta_2$ adrenergic receptor. Examples of suitable non-selective beta adrenergic receptor antagonists include timolol, levobunolol, carteolol and metipranolol.

[0037] In yet another aspect, the aqueous humor modulating agent is an adrenergic agonists. Adrenergic agonists typically bind to and stimulate adrenergic receptors, causing responses similar to those of adrenaline and noradrenaline, including the inhibition of aqueous humor production. In general, any adrenergic receptor agonists capable of lowering IOP by altering the production, inflow or outflow of aqueous humor may be used in the composition. In one embodiment, the adrenergic receptor agonist is alpha-2 adrenergic receptor agonists. By way of example, suitable alpha-2 adrenergic receptor agonists include apraclonidine and brimonidine. In a further embodiment, the adrenergic receptor agonist is epinephrine. In some embodiments, the adrenergic receptor agonists may be a pharmaceutically acceptable salt of epinephrine such as epinephryl borate, epinephrine bydrochloride or epinephrine bitartate. In other embodiments, the adrenergic receptor agonist may be a prodrug of epinephrine such as dipivefrin.

[0038] In still another aspect, the aqueous humor modulating agent is a mitotic. Generally speaking, miotics are divided into two categories: direct and indirect cholinergic agents. Irrespective of their classification, mitotic agents generally lower IOP by stimulating smooth muscle muscarinic receptors, causing a widening of the trabecular meshwork to increase aqueous humor outflow. By way of example, suitable direct cholinergic agents include pilocarpine, pilocarpine hydrochloride, and carbachol. Examples of suitable indirect cholinergic agents include echothiophate iodide, echothiophate, demacarium, and physostigmine.

[0039] In a further aspect, the aqueous humor modulating agent is a carbonic anhydrase inhibitor. CA is an enzyme involved in producing bicarbonate,

which is required for aqueous humor production by the ciliary tissues in the eye. By inhibiting CA, accordingly, production of aqueous humor is substantially reduced. Generally speaking, the CA inhibitor may inhibit any isomer of the metalloprotein enzyme that catalyzes the interconversion of CO_2 and H_2CO_3 ($CO_2 + O_2 \rightarrow HCO_2^- + H^+$). Typically, however, the CA inhibitor will inhibit the CAI, CAII or CAIV isoform. Examples of suitable CA inhibitors include acetazolamide, methazolamide, dorzolamide hydrochloride ophthalmic solution, dorzolamide hydrochloride-timolol maleate ophthalmic solution, brinzolamide hydrochloride, dorzolamide, and brinzolamide.

[0040] Other aqueous humor modulating agents that may be used to reduce IOP include cannabinoids drug class, for example, anandamine; selective and unselective PKC inhibitors drug class; rho kinase inhibitors drug class; and combinations thereof; corticosteroid receptor antagonists; selective and nonselective dopamine DA-1 agonists; TNF antagonists; somatostatin selective sst4 agonists; angiotensin II antagonists; thyroxine; adenosine 3 antagonists, vacuolar proton ATPase inhibitors such as bafilomycin; sodium hydrogen antiporter inhibitors; chloride anion exchanger inhibitors; and combinations thereof.

[0041] It is contemplated that the composition may include more than one aqueous humor modulating agent. Generally speaking, combinations are selected so as to include agents that have different modes of action and work on different receptor sites or enzymes, but that do not antagonize one another. By way of illustrative example, an ineffective combination may include brimonidine with a beta blocker and brimonidine with epinephrine. Both brimonidine and beta blockers suppress the formation of cAMP in the ciliary epithelium, while epinephrine upregulates the adenyl cyclase enzyme that brimonidine indirectly inhibits. By way of further illustrative example, an effective combination may include a beta blocker with a cholinergic agent or a beta blocker with a CA inhibitor, as both combinations include agents that target different receptor sites or enzymes.

Routes of Administration

[0042] Generally speaking, the AQP modulating agent and aqueous humor modulating agents useful in the practice of the present invention can be formulated into pharmaceutical compositions and administered separately, either simultaneously or sequentially. Alternatively, the AQP modulating agent and aqueous humor

modulating agent can be formulated into a single composition comprising both agents. Irrespective of whether both agents are formulated into a single composition or formulated with each agent in a separate composition, the composition may be administered by any means that will deliver a therapeutically effective dose of both agents, as detailed herein or as otherwise known in the art. For example, formulation of agents is discussed in Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania (1975), and Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y. (1980).

[0043] In one aspect, the composition is administered directly to the eye by any means known in the art such as in a solution, cream, ointment, emulsion, suspension and slow release formulations. Administration of a composition to the eye generally results in direct contact of the agents with the cornea, through which at least a portion of the administered agents pass. In general, the composition has an effective residence time in the eye of about 2 to about 24 hours, more typically about 4 to about 24 hours and most typically about 6 to about 24 hours.

[0044] A composition of the invention can illustratively take the form of a liquid where the agents are present in solution, in suspension or both. Typically when the composition is administered as a solution or suspension a first portion of the agent is present in solution and a second portion of the agent is present in particulate form, in suspension in a liquid matrix. In some embodiments, a liquid composition may include a gel formulation. In other embodiments, the liquid composition is aqueous. Alternatively, the composition can take the form of an ointment.

[0045] In one embodiment, the composition is an aqueous solution, suspension or solution/suspension, which can be presented in the form of eye drops. By means of a suitable dispenser, a desired dosage of each agent can be metered by administration of a known number of drops into the eye. For example, for a drop volume of 25 μl, administration of 1-6 drops will deliver 25-150 μl of the composition. Aqueous compositions of the invention typically contain from about 0.01% to about 50%, more typically about 0.1% to about 20%, still more typically about 0.2% to about 10%, and most typically about 0.5% to about 5%, weight/volume of the AQP modulating agent and aqueous humor modulating agent.

[0046] Generally speaking, aqueous compositions of the invention have ophthalmically acceptable pH and osmolality. "Ophthalmically acceptable" with respect to a formulation, composition or ingredient typically means having no persistent detrimental effect on the treated eye or the functioning thereof, or on the general health of the subject being treated. It will be recognized that transient effects such as minor irritation or a "stinging" sensation are common with topical ophthalmic administration of agents and the existence of such transient effects is not inconsistent with the formulation, composition or ingredient in question being "ophthalmically acceptable" as detailed herein. But formulations, compositions and ingredients employed in the present invention are those that generally cause no substantial detrimental effect, even of a transient nature.

[0047] In an aqueous suspension or solution/suspension composition, the agent can be present predominantly in the form of nanoparticles, *i.e.*, solid particles smaller than about 1000 nm in their longest dimension. A benefit of this composition is more rapid release of the agent, and therefore more complete release during the residence time of the composition in a treated eye than occurs with larger particle size. Another benefit is reduced potential for eye irritation by comparison with larger particle size. Reduced eye irritation in turn leads to a reduced tendency for loss of the composition from the treated eye by lacrimation, which is stimulated by such irritation.

[0048] In a related composition, the agent typically has a D_{90} particle size of about 10 to about 2000 nm, wherein about 25% to 100% by weight of the particles are nanoparticles. "D_{90}" is a linear measure of diameter having a value such that 90% by volume of particles in the composition, in the longest dimension of the particles, are smaller than that diameter. For practical purposes a determination of D_{90} based on 90% by weight rather than by volume is generally suitable.

[0049] In one composition, substantially all of the agent particles in the composition are smaller than 100 nm, *i.e.*, the percentage by weight of nanoparticles is 100% or close to 100%. Generally speaking, the average particle size of the agent in this embodiment is typically about 100 to about 800 nm, more typically about 150 to about 600 nm, and even more typically, about 200 to about 400 nm. The agent can be in crystalline or amorphous form in the nanoparticles. Processes for preparing nanoparticles that involve milling or grinding typically provide the agent

in crystalline form, whereas processes that involve precipitation from solution typically provide the agent in amorphous form.

[0050] The ophthalmic composition in some embodiments can be an aqueous suspension of an agent of low water solubility, wherein typically the agent is present predominantly or substantially entirely in nanoparticulate form. Without being bound by theory, it is believed that release of the agent from nanoparticles is significantly faster than from a typical "micronized" composition having a D_{90} particle size of, for example, about 10,000 nm or greater.

[0051] In another embodiment, an aqueous suspension composition of the invention can comprise a first portion of the agent in nanoparticulate form, to promote relatively rapid release, and a second portion of the agent having a D_{90} particle size of about 10,000 nm or greater, that can provide a depot or reservoir of the agent in the treated eye for release over a period of time, for example about 2 to about 24 hours, more typically about 2 to about 12 hours, to promote sustained therapeutic effect and permit a reduced frequency of administration.

[0052] In still another embodiment, an aqueous suspension can contain one or more polymers as suspending agents. Useful polymers include water-soluble polymers such as cellulosic polymers, *e.g.*, hydroxypropyl methylcellulose, and water-insoluble polymers such as cross-linked carboxyl-containing polymers.

[0053] The composition can be an *in situ* gellable aqueous solution, suspension or solution/suspension having excipients substantially as disclosed in U.S. Patent No. 5,192,535, comprising about 0.1% to about 6.5%, typically about 0.5% to about 4.5%, by weight, based on the total weight of the composition, of one or more cross-linked carboxyl-containing polymers. Such an aqueous suspension is typically sterile and has an osmolality of about 10 to about 400 mOsM, typically about 100 to about 250 mOsM, a pH of about 3 to about 6.5, typically about 4 to about 6, and an initial viscosity, when administered to the eye, of about 1000 to about 30,000 cPs, as measured at 25°C using a Brookfield Digital LVT viscometer with #25 spindle and 13R small sample adapter at 12 rpm. More typically the initial viscosity is about 5000 to about 20,000 cPs. The polymer component has an average particle size not greater than about 50 μm, typically not greater than about 30 μm, more typically not greater than about 20 μm, and most typically about 1 μm to about 5 μm, in equivalent spherical diameter, and is lightly cross-linked to a degree

such that, upon contact with tear fluid in the eye, which has a typical pH of about 7.2 to about 7.4, the viscosity of the suspension rapidly increases, to form a gel. This formation of a gel enables the composition to remain in the eye for a prolonged period without loss by lacrimal drainage.

[0054] Suitable carboxyl-containing polymers for use in this composition are prepared from one or more carboxyl-containing monoethylenically unsaturated monomers such as acrylic, methacrylic, ethacrylic, crotonic, angelic, tiglic, α-butylcrotonic, α-phenylacrylic, α-benzylacrylic, α-cyclohexylacrylic, cinnamic, coumaric and umbellic acids, most typically acrylic acid. The polymers are cross-linked by using less than about 5%, typically about 0.1% to about 5%, more typically about 0.2% to about 1%, by weight of one or more polyfunctional cross-linking agents such as non-polyalkenyl polyether difunctional cross-linking monomers, *e.g.*, divinyl glycol. Other suitable cross-linking agents illustratively include 2,3-dihydroxyhexa-1,5-diene, 2,5-dimethylhexa-1,5-diene, divinylbenzene, N,N-diallylacrylamide and N,N-diallylmethacrylamide. Divinyl glycol is typically employed. Polyacrylic acid cross-linked with divinyl glycol is called polycarbophil. A polymer system containing polycarbophil is commercially available under the trademark DuraSite® of InSite Vision Inc., Alameda, CA, as a sustained-release topical ophthalmic delivery system.

[0055] In another formulation, the composition can be an *in situ* gellable aqueous solution, suspension or solution/suspension having excipients substantially as disclosed in U.S. Patent No. 4,861,760, comprising about 0.1% to about 2% by weight of a polysaccharide that gels when it contacts an aqueous medium having the ionic strength of tear fluid. One such polysaccharide is gellan gum. This composition can be prepared by a procedure substantially as disclosed in U.S. Patent No. 4,861,760.

[0056] In yet another formulation, the composition can be an *in situ* gellable aqueous solution, suspension or solution/suspension having excipients substantially as disclosed in U.S. Patent No. 5,587,175, comprising about 0.2% to about 3%, typically about 0.5% to about 1%, by weight of a gelling polysaccharide, typically selected from gellan gum, alginate gum and chitosan, and about 1% to about 50% of a water-soluble film-forming polymer, typically selected from alkylcelluloses (*e.g.*, methylcellulose, ethylcellulose), hydroxyalkylcelluloses (*e.g.*,

hydroxyethylcellulose, hydroxypropyl methylcellulose), hyaluronic acid and salts thereof, chondroitin sulfate and salts thereof, polymers of acrylamide, acrylic acid and polycyanoacrylates, polymers of methyl methacrylate and 2-hydroxyethyl methacrylate, polydextrose, cyclodextrins, polydextrin, maltodextrin, dextran, polydextrose, gelatin, collagen, natural gums (*e.g.*, xanthan, locust bean, acacia, tragacanth and carrageenan gums and agar), polygalacturonic acid derivatives (*e.g.*, pectin), polyvinyl alcohol, polyvinylpyrrolidone and polyethylene glycol. The composition can optionally contain a gel-promoting counterion such as calcium in latent form, for example encapsulated in gelatin. This composition can be prepared by a procedure substantially as disclosed in U.S. Patent No. 5,587,175.

[0057] In a further formulation, the composition can be an *in situ* gellable aqueous solution, suspension or solution/suspension having excipients substantially as disclosed in European Patent No. 09/424,043, comprising about 0.1% to about 5% of a carrageenan gum. In this embodiment, a carrageenan having no more than 2 sulfate groups per repeating disaccharide unit is typical, including kappa-carrageenan, having 18-25% ester sulfate by weight, iota-carrageenan, having 25-34% ester sulfate by weight, and mixtures thereof.

[0058] In still another particular formulation, the composition comprises an ophthalmically acceptable mucoadhesive polymer, selected for example from carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, acrylic acid/butyl acrylate copolymer, sodium alginate and dextran.

[0059] In another composition, the agent is solubilized at least in part by an ophthalmically acceptable solubilizing agent. The term "solubilizing agent" generally includes agents that result in formation of a micellar solution or a true solution of the agent. Certain ophthalmically acceptable nonionic surfactants, for example polysorbate 80, can be useful as solubilizing agents, as can ophthalmically acceptable glycols, polyglycols, *e.g.*, polyethylene glycol 400, and glycol ethers.

[0060] A class of solubilizing agents suitable for use in solution and solution/suspension compositions of the invention is the cyclodextrins. Suitable cyclodextrins can be selected from α-cyclodextrin, α-cyclodextrin, α-cyclodextrin, alkylcyclodextrins (*e.g.*, methyl-α-cyclodextrin, dimethyl-α-cyclodextrin, diethyl-α-cyclodextrin), hydroxyalkylcyclodextrins (*e.g.*, hydroxyethyl-α-cyclodextrin,

hydroxypropyl-α-cyclodextrin), carboxyalkylcyclodextrins (*e.g.*, carboxymethyl-α-cyclodextrin), sulfoalkylether cyclodextrins (*e.g.*, sulfobutylether-α-cyclodextrin), and the like. Ophthalmic applications of cyclodextrins have been reviewed by Rajewski & Stella (1996), Journal of Pharmaceutical Sciences, 85, 1154, at pages 1155-1159. If desired, complexation of an agent by a cyclodextrin can be increased by addition of a water-soluble polymer such as carboxymethylcellulose, hydroxypropyl methylcellulose or polyvinylpyrrolidone, as described by Loftsson (1998), Pharmazie, 53, 733-740.

[0061] In some embodiments, one or more ophthalmically acceptable pH adjusting agents or buffering agents can be included in a composition of the invention, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an ophthalmically acceptable range.

[0062] In another embodiment, one or more ophthalmically acceptable salts can be included in the composition in an amount required to bring osmolality of the composition into an ophthalmically acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate. Optionally one or more ophthalmically acceptable acids having at least two dissociable hydrogen groups can be included in a polymer-containing composition as interactive agents to retard release of the agent through inhibition of erosion of the polymer, as disclosed in International Patent Publication No. WO 95/03784. Acids useful as interactive agents include boric, lactic, orthophosphoric, citric, oxalic, succinic, tartaric and formic glycerophosphoric acids.

[0063] In still another embodiment, an ophthalmically acceptable xanthine derivative such as caffeine, theobromine or theophylline can be included in the composition, substantially as disclosed in U.S. Patent No. 4,559,343, to reduce ocular discomfort associated with administration of the composition.

[0064] In yet another embodiment, one or more ophthalmically acceptable preservatives can be included in the composition to inhibit microbial activity. Suitable preservatives include mercury-containing substances such as merfen and thiomersal; stabilized chlorine dioxide; and quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride.

[0065] In a further embodiment, one or more ophthalmically acceptable surfactants, typically nonionic surfactants, can be included in the composition to enhance physical stability or for other purposes. Suitable nonionic surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40.

[0066] In another embodiment, one or more antioxidants can be included in the composition to enhance chemical stability where required. Suitable antioxidants include ascorbic acid and sodium metabisulfite.

[0067] In still another embodiment, one or more ophthalmic lubricating agents can optionally be included in the composition to promote lacrimation or as a "dry eye" medication. Such agents include polyvinyl alcohol, methylcellulose, hydroxypropyl methylcellulose, polyvinylpyrrolidone, *etc.*

[0068] Aqueous suspension compositions of the invention can be packaged in single-dose non-reclosable containers. Such containers can maintain the composition in a sterile condition and thereby eliminate need for preservatives such as mercury-containing preservatives, which can sometimes cause irritation and sensitization of the eye. Alternatively, multiple-dose reclosable containers can be used, in which case it is typical to include a preservative in the composition.

[0069] As a further alternative, the composition can take the form of a solid article that can be inserted between the eye and eyelid or in the conjunctival sac, where it releases the agent as described, for example, in U.S. Patent No. 3,863,633 and U.S. Patent No. 3,868,445, both to Ryde & Ekstedt, incorporated herein by reference. Release is to the lacrimal fluid that bathes the surface of the cornea, or directly to the cornea itself, with which the solid article is generally in intimate contact. Solid articles suitable for implantation in the eye in such fashion are generally composed primarily of polymers and can be biodegradable or non-biodegradable. Biodegradable polymers that can be used in preparation of ocular

implants carrying an AQP modulating agent or aqueous humor modulating agent in accordance with the present invention include without restriction aliphatic polyesters such as polymers and copolymers of poly(glycolide), poly(lactide), poly(α-caprolactone), poly(hydroxybutyrate) and poly(hydroxyvalerate), polyamino acids, polyorthoesters, polyanhydrides, aliphatic polycarbonates and polyether lactones. Suitable non-biodegradable polymers include silicone elastomers.

[0070] In another aspect of the invention, the composition is not administered directly to the eye. By way of example, such a composition can be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired.

[0071] Solid dosage forms for oral administration can include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the agents of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered *per os*, an agent can be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets can contain a controlled-release formulation as can be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. In the case of capsules, tablets, and pills, the dosage forms can also comprise buffering agents such as sodium citrate, magnesium or calcium carbonate or bicarbonate. Tablets and pills can additionally be prepared with enteric coatings.

[0072] Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

[0073] The term parenteral includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated

according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

[0074] For therapeutic purposes, formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions can be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. A contemplated therapeutic compound can be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride solution, or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Dosages

[0075] In general, the actual effective amounts of AQP modulating agent can and will vary according to the specific composition being utilized, the mode of administration and the age, weight and condition of the subject. Dosages for a particular individual subject can be determined by one of ordinary skill in the art using conventional considerations.

[0076] By way of example, when the AQP modulating agent is an angiotensin-converting enzyme inhibitor administered orally, suitable dosages and dosing regimens are shown in Table 1 for several inhibitors.

Table 1- Dosages of Oral Angiotensin-Converting Enzyme Inhibitors

Agent	Target Dose
Benazepril (Lotensin/Novartis)	20-40 mg QD or divided BID
Captopril (Capoten/Bristol-Myers Squibb)	HTN: 25-150 mg BID-TID HF: 50-100 mg TID Post-MI: 50 mg TID DN: 25 mg TID
Enalapril (Vasotec/Merck & Co.)	HTN: 10-40 mg QD or divided HID HF: 2.5-20 mg divided BID ALVD: 20 mg divided BID
Fosinopril (monopril/Bristol-Myers Squibb)	HTN, HF: 20-40 mg QD or divided BID
Lisinopril (Prinivil/Merck, Zestril/Zeneca)	HTN: 20-40 mg QD HF: 5-20 mg QD Post-MI: 10 mg QD
Moexipril (univasc/Schwarz Pharma)	7.5-30 mg QD or divided BID
Quinapril (Accupril/Parke-Davis)	HTN: 20-80 mg QD or divided BID HF: 20-40 mg divided BID
Ramipril (Altace/Hoechst Marion Roussel)	HTN: 2.5-20 mg QD or divided BID HF: 5 mg BID
Trandolapril (Mavik/Knoll)	2-4 mg QD or divided BID

[0077] By way of further example, when the AQP modulating agent is arginine vasopressin, the amount administered daily is typically from about 0.5 to about 10 micrograms/kilogram body weight per day.

[0078] By way of still further example, when the AQP modulating agent is atrial natriuretic peptide, the amount administered daily is typically from about 0.5 to about 10 micrograms/kilogram body weight per day.

[0079] Moreover, the actual effective amounts of the aqueous humor modulating agent can and will vary according to the specific composition being utilized, the mode of administration and the age, weight and condition of the subject. Dosages for a particular individual subject can be determined by one of ordinary skill in the art using conventional considerations.

[0080] By way of example, when the aqueous humor modulating agent is a beta blocker, adrenergic agonist, CA inhibitor, cholinergic agonist, prostaglandin analog, or alpha agonist, suitable dosages and dosing regimens are shown in Table 2 for several agents belonging to each class.

TABLE 2			
Generic Name	**Brand Name**	**Strength**	**Administration**
Beta Blockers			
Betaxolo	Betoptic	0.25-0.5%	BID
Carteolol	Ocupress	1%	BID
Levobunolol	Betagan	0.25-0.5%	QD-BID
Metipranolol	OptiPranolol	0.30%	BID
Timolol	Timpotic/Betimol	0.25-0.5%	BID (gel QD)
Levobetaxolol	Betaxon	0.50%	BID
Adrenergic Agonists			
Epinephrine	Epifren	0.1-0.2%	QD-BID
Dipivefrin	Propine	0.10%	Q12h
Oral Carbonic Anhydrase Inhibitors			
Acetazolamide	Diamox	250/500 mg (SR)	QID/BID for SR
Methazolamide	Neptazane	25-100 mg	TID
Cholinergic Agonists			
Pilocarpine	Isopto Carpine/Pilocar/Pilostat	0.25-10%	BID-QID
Carbachol	Isopto Carbachol/carboptic	0.75-3%	TID
Demacarium	Humursol	0.125-0.25%	BID
Echothiophate Iodide	Phospholine Iodide	0.03-0.25%	BID
Physostigmine	Isopto Eserine	0.25-0.5%	TID-QID
Topical Carbonic Anhydrase Inhibitors			
Dorzolamide	Trusopt	2%	TID
Brinzolamide	Azopt	1%	TID
Prostaglandin Analogs			
Latanoprost	Xalatan	0.01%	QD (in evening)
Unoprostone	Rescula	0.15%	BID
Bimatoprost	Lumigan	0.03%	QD (in evening)
Travoprost	Travatan	0.004%	QD (in evening)
Alpha Agonists			
Apraclonidine	Iopidine	0.5-1%	TID
Brimonidine	Alphagan	0.20%	TID
Combinations			
Dorzolamide/Timolol	Cosopt	2%/0.5%	BID

Combination Therapies

[0081] Generally speaking, it is contemplated that the composition employed in the practice of the invention may include one or more of any of the aquaporin modulating agents detailed above in combination with one or more of any of the aqueous humor modulating agents detailed above. By way of a non limiting

example, Table 3 details a number of suitable combinations that are useful in the methods and compositions of the current invention. The combination may also include an isomer, a pharmaceutically acceptable salt, ester, or prodrug of any of the aquaporin modulating agents or aqueous humor modulating agents listed in Table 3.

TABLE NO. 3	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
carbonic anhydrase inhibitor	prostaglandin
carbonic anhydrase inhibitor	prostaglandin analog
carbonic anhydrase inhibitor	beta adrenergic antagonist
carbonic anhydrase inhibitor	prostaglandin FP receptor antagonist
carbonic anhydrase inhibitor	adrenergic agonist
carbonic anhydrase inhibitor	cholinergic agonist
carbonic anhydrase inhibitor	carbonic anhydrase inhibitor
angiotensin converting enzyme inhibitor	prostaglandin
angiotensin converting enzyme inhibitor	prostaglandin analog
angiotensin converting enzyme inhibitor	beta adrenergic antagonist
angiotensin converting enzyme inhibitor	prostaglandin FP receptor antagonist
angiotensin converting enzyme inhibitor	adrenergic agonist
angiotensin converting enzyme inhibitor	cholinergic agonist
angiotensin converting enzyme inhibitor	carbonic anhydrase inhibitor
protein kinase C activator	prostaglandin
protein kinase C activator	prostaglandin analog
protein kinase C activator	beta adrenergic antagonist
protein kinase C activator	prostaglandin FP receptor antagonist
protein kinase C activator	adrenergic agonist
protein kinase C activator	cholinergic agonist
protein kinase C activator	carbonic anhydrase inhibitor
protein kinase A inhibitor	prostaglandin
protein kinase A inhibitor	prostaglandin analog
protein kinase A inhibitor	beta adrenergic antagonist
protein kinase A inhibitor	prostaglandin FP receptor antagonist
protein kinase A inhibitor	adrenergic agonist
protein kinase A inhibitor	cholinergic agonist
protein kinase A inhibitor	carbonic anhydrase inhibitor
vasoactive peptide	prostaglandin
vasoactive peptide	prostaglandin analog
vasoactive peptide	beta adrenergic antagonist
vasoactive peptide	prostaglandin FP receptor antagonist

TABLE NO. 3	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
vasoactive peptide	adrenergic agonist
vasoactive peptide	cholinergic agonist
vasoactive peptide	carbonic anhydrase inhibitor
adenylate cyclase inhibitor	prostaglandin
adenylate cyclase inhibitor	prostaglandin analog
adenylate cyclase inhibitor	beta adrenergic antagonist
adenylate cyclase inhibitor	prostaglandin FP receptor antagonist
adenylate cyclase inhibitor	adrenergic agonist
adenylate cyclase inhibitor	cholinergic agonist
adenylate cyclase inhibitor	carbonic anhydrase inhibitor
tetraethylammonium	prostaglandin
tetraethylammonium	prostaglandin analog
tetraethylammonium	beta adrenergic antagonist
tetraethylammonium	prostaglandin FP receptor antagonist
tetraethylammonium	adrenergic agonist
tetraethylammonium	cholinergic agonist
tetraethylammonium	carbonic anhydrase inhibitor
colchicine	prostaglandin
colchicine	prostaglandin analog
colchicine	beta adrenergic antagonist
colchicine	prostaglandin FP receptor antagonist
colchicine	adrenergic agonist
colchicine	cholinergic agonist
colchicine	carbonic anhydrase inhibitor
vinca alkaloid	prostaglandin
vinca alkaloid	prostaglandin analog
vinca alkaloid	beta adrenergic antagonist
vinca alkaloid	prostaglandin FP receptor antagonist
vinca alkaloid	adrenergic agonist
vinca alkaloid	cholinergic agonist
vinca alkaloid	carbonic anhydrase inhibitor
rhizoxin	prostaglandin
rhizoxin	prostaglandin analog
rhizoxin	beta adrenergic antagonist
rhizoxin	prostaglandin FP receptor antagonist
rhizoxin	adrenergic agonist
rhizoxin	cholinergic agonist
rhizoxin	carbonic anhydrase inhibitor
estramustine	prostaglandin
estramustine	prostaglandin analog
estramustine	beta adrenergic antagonist
estramustine	prostaglandin FP receptor antagonist
estramustine	adrenergic agonist
estramustine	cholinergic agonist
estramustine	carbonic anhydrase inhibitor
nocodazole	prostaglandin

TABLE NO. 3	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
nocodazole	prostaglandin analog
nocodazole	beta adrenergic antagonist
nocodazole	prostaglandin FP receptor antagonist
nocodazole	adrenergic agonist
nocodazole	cholinergic agonist
nocodazole	carbonic anhydrase inhibitor
erbuluzole	prostaglandin
erbuluzole	prostaglandin analog
erbuluzole	beta adrenergic antagonist
erbuluzole	prostaglandin FP receptor antagonist
erbuluzole	adrenergic agonist
erbuluzole	cholinergic agonist
erbuluzole	carbonic anhydrase inhibitor
tubulozole	prostaglandin
tubulozole	prostaglandin analog
tubulozole	beta adrenergic antagonist
tubulozole	prostaglandin FP receptor antagonist
tubulozole	adrenergic agonist
tubulozole	cholinergic agonist
tubulozole	carbonic anhydrase inhibitor
cytochalasin D	prostaglandin
cytochalasin D	prostaglandin analog
cytochalasin D	beta adrenergic antagonist
cytochalasin D	prostaglandin FP receptor antagonist
cytochalasin D	adrenergic agonist
cytochalasin D	cholinergic agonist
cytochalasin D	carbonic anhydrase inhibitor
diacylglycerol mimic	prostaglandin
diacylglycerol mimic	prostaglandin analog
diacylglycerol mimic	beta adrenergic antagonist
diacylglycerol mimic	prostaglandin FP receptor antagonist
diacylglycerol mimic	adrenergic agonist
diacylglycerol mimic	cholinergic agonist
diacylglycerol mimic	carbonic anhydrase inhibitor
phorbol ester	prostaglandin
phorbol ester	prostaglandin analog
phorbol ester	beta adrenergic antagonist
phorbol ester	prostaglandin FP receptor antagonist
phorbol ester	adrenergic agonist
phorbol ester	cholinergic agonist
phorbol ester	carbonic anhydrase inhibitor

[0082] In a further embodiment, Table 4 details a number of suitable combinations that are useful in the methods and compositions of the current invention. The combination may also include an isomer, a pharmaceutically

acceptable salt, ester, or prodrug of any of the aquaporin modulating agents or aqueous humor modulating agents listed in Table 4.

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
cytochalasin D	prostaglandin A
cytochalasin D	prostaglandin B
cytochalasin D	prostaglandin D
cytochalasin D	prostaglandin E
cytochalasin D	prostaglandin F
cytochalasin D	latanaprost
cytochalasin D	bimatoprost
cytochalasin D	unoprostone
cytochalasin D	travoprost
cytochalasin D	betaxolol
cytochalasin D	carteolol
cytochalasin D	levobunolol
cytochalasin D	metipranolol
cytochalasin D	timolol
cytochalasin D	levobetaxolol
cytochalasin D	epinephrine
cytochalasin D	dipivefrin
cytochalasin D	pilocarpine
cytochalasin D	pilocarpine hydrochloride
cytochalasin D	carbachol
cytochalasin D	demacarium
cytochalasin D	echothiophate iodine
cytochalasin D	physostigmine
cytochalasin D	acetazolamide
cytochalasin D	methazolamide
cytochalasin D	dorzolamide
cytochalasin D	brinzolamide
acetazolamide	prostaglandin A
acetazolamide	prostaglandin B
acetazolamide	prostaglandin D
acetazolamide	prostaglandin E
acetazolamide	prostaglandin F
acetazolamide	latanaprost
acetazolamide	bimatoprost
acetazolamide	unoprostone
acetazolamide	travoprost
acetazolamide	betaxolol
acetazolamide	carteolol
acetazolamide	levobunolol
acetazolamide	metipranolol
acetazolamide	timolol
acetazolamide	levobetaxolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
acetazolamide	epinephrine
acetazolamide	dipivefrin
acetazolamide	pilocarpine
acetazolamide	pilocarpine hydrochloride
acetazolamide	carbachol
acetazolamide	demacarium
acetazolamide	echothiophate iodine
acetazolamide	physostigmine
acetazolamide	acetazolamide
acetazolamide	methazolamide
acetazolamide	dorzolamide
acetazolamide	brinzolamide
methazolamide	prostaglandin A
methazolamide	prostaglandin B
methazolamide	prostaglandin D
methazolamide	prostaglandin E
methazolamide	prostaglandin F
methazolamide	latanaprost
methazolamide	bimatoprost
methazolamide	unoprostone
methazolamide	travoprost
methazolamide	betaxolol
methazolamide	carteolol
methazolamide	levobunolol
methazolamide	metipranolol
methazolamide	timolol
methazolamide	levobetaxolol
methazolamide	epinephrine
methazolamide	dipivefrin
methazolamide	pilocarpine
methazolamide	pilocarpine hydrochloride
methazolamide	carbachol
methazolamide	demacarium
methazolamide	echothiophate iodine
methazolamide	physostigmine
methazolamide	acetazolamide
methazolamide	methazolamide
methazolamide	dorzolamide
methazolamide	brinzolamide
enalapril	prostaglandin A
enalapril	prostaglandin B
enalapril	prostaglandin D
enalapril	prostaglandin E
enalapril	prostaglandin F
enalapril	latanaprost
enalapril	bimatoprost

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
enalapril	unoprostone
enalapril	travoprost
enalapril	betaxolol
enalapril	carteolol
enalapril	levobunolol
enalapril	metipranolol
enalapril	timolol
enalapril	levobetaxolol
enalapril	epinephrine
enalapril	dipivefrin
enalapril	pilocarpine
enalapril	pilocarpine hydrochloride
enalapril	carbachol
enalapril	demacarium
enalapril	echothiophate iodine
enalapril	physostigmine
enalapril	acetazolamide
enalapril	methazolamide
enalapril	dorzolamide
enalapril	brinzolamide
benazepril	prostaglandin A
benazepril	prostaglandin B
benazepril	prostaglandin D
benazepril	prostaglandin E
benazepril	prostaglandin F
benazepril	latanaprost
benazepril	bimatoprost
benazepril	unoprostone
benazepril	travoprost
benazepril	betaxolol
benazepril	carteolol
benazepril	levobunolol
benazepril	metipranolol
benazepril	timolol
benazepril	levobetaxolol
benazepril	epinephrine
benazepril	dipivefrin
benazepril	pilocarpine
benazepril	pilocarpine hydrochloride
benazepril	carbachol
benazepril	demacarium
benazepril	echothiophate iodine
benazepril	physostigmine
benazepril	acetazolamide
benazepril	methazolamide
benazepril	dorzolamide

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
benazepril	brinzolamide
captopril	prostaglandin A
captopril	prostaglandin B
captopril	prostaglandin D
captopril	prostaglandin E
captopril	prostaglandin F
captopril	latanaprost
captopril	bimatoprost
captopril	unoprostone
captopril	travoprost
captopril	betaxolol
captopril	carteolol
captopril	levobunolol
captopril	metipranolol
captopril	timolol
captopril	levobetaxolol
captopril	epinephrine
captopril	dipivefrin
captopril	pilocarpine
captopril	pilocarpine hydrochloride
captopril	carbachol
captopril	demacarium
captopril	echothiophate iodine
captopril	physostigmine
captopril	acetazolamide
captopril	methazolamide
captopril	dorzolamide
captopril	brinzolamide
fosinopril	prostaglandin A
fosinopril	prostaglandin B
fosinopril	prostaglandin D
fosinopril	prostaglandin E
fosinopril	prostaglandin F
fosinopril	latanaprost
fosinopril	bimatoprost
fosinopril	unoprostone
fosinopril	travoprost
fosinopril	betaxolol
fosinopril	carteolol
fosinopril	levobunolol
fosinopril	metipranolol
fosinopril	timolol
fosinopril	levobetaxolol
fosinopril	epinephrine
fosinopril	dipivefrin
fosinopril	pilocarpine

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
fosinopril	pilocarpine hydrochloride
fosinopril	carbachol
fosinopril	demacarium
fosinopril	echothiophate iodine
fosinopril	physostigmine
fosinopril	acetazolamide
fosinopril	methazolamide
fosinopril	dorzolamide
fosinopril	brinzolamide
lisinopril	prostaglandin A
lisinopril	prostaglandin B
lisinopril	prostaglandin D
lisinopril	prostaglandin E
lisinopril	prostaglandin F
lisinopril	latanaprost
lisinopril	bimatoprost
lisinopril	unoprostone
lisinopril	travoprost
lisinopril	betaxolol
lisinopril	carteolol
lisinopril	levobunolol
lisinopril	metipranolol
lisinopril	timolol
lisinopril	levobetaxolol
lisinopril	epinephrine
lisinopril	dipivefrin
lisinopril	pilocarpine
lisinopril	pilocarpine hydrochloride
lisinopril	carbachol
lisinopril	demacarium
lisinopril	echothiophate iodine
lisinopril	physostigmine
lisinopril	acetazolamide
lisinopril	methazolamide
lisinopril	dorzolamide
lisinopril	brinzolamide
moexipril	prostaglandin A
moexipril	prostaglandin B
moexipril	prostaglandin D
moexipril	prostaglandin E
moexipril	prostaglandin F
moexipril	latanaprost
moexipril	bimatoprost
moexipril	unoprostone
moexipril	travoprost
moexipril	betaxolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
moexipril	carteolol
moexipril	levobunolol
moexipril	metipranolol
moexipril	timolol
moexipril	levobetaxolol
moexipril	epinephrine
moexipril	dipivefrin
moexipril	pilocarpine
moexipril	pilocarpine hydrochloride
moexipril	carbachol
moexipril	demacarium
moexipril	echothiophate iodine
moexipril	physostigmine
moexipril	acetazolamide
moexipril	methazolamide
moexipril	dorzolamide
moexipril	brinzolamide
quinapril	prostaglandin A
quinapril	prostaglandin B
quinapril	prostaglandin D
quinapril	prostaglandin E
quinapril	prostaglandin F
quinapril	latanaprost
quinapril	bimatoprost
quinapril	unoprostone
quinapril	travoprost
quinapril	betaxolol
quinapril	carteolol
quinapril	levobunolol
quinapril	metipranolol
quinapril	timolol
quinapril	levobetaxolol
quinapril	epinephrine
quinapril	dipivefrin
quinapril	pilocarpine
quinapril	pilocarpine hydrochloride
quinapril	carbachol
quinapril	demacarium
quinapril	echothiophate iodine
quinapril	physostigmine
quinapril	acetazolamide
quinapril	methazolamide
quinapril	dorzolamide
quinapril	brinzolamide
ramipril	prostaglandin A
ramipril	prostaglandin B

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
ramipril	prostaglandin D
ramipril	prostaglandin E
ramipril	prostaglandin F
ramipril	latanaprost
ramipril	bimatoprost
ramipril	unoprostone
ramipril	travoprost
ramipril	betaxolol
ramipril	carteolol
ramipril	levobunolol
ramipril	metipranolol
ramipril	timolol
ramipril	levobetaxolol
ramipril	epinephrine
ramipril	dipivefrin
ramipril	pilocarpine
ramipril	pilocarpine hydrochloride
ramipril	carbachol
ramipril	demacarium
ramipril	echothiophate iodine
ramipril	physostigmine
ramipril	acetazolamide
ramipril	methazolamide
ramipril	dorzolamide
ramipril	brinzolamide
tandolapril	prostaglandin A
tandolapril	prostaglandin B
tandolapril	prostaglandin D
tandolapril	prostaglandin E
tandolapril	prostaglandin F
tandolapril	latanaprost
tandolapril	bimatoprost
tandolapril	unoprostone
tandolapril	travoprost
tandolapril	betaxolol
tandolapril	carteolol
tandolapril	levobunolol
tandolapril	metipranolol
tandolapril	timolol
tandolapril	levobetaxolol
tandolapril	epinephrine
tandolapril	dipivefrin
tandolapril	pilocarpine
tandolapril	pilocarpine hydrochloride
tandolapril	carbachol
tandolapril	demacarium

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
tandolapril	echothiophate iodine
tandolapril	physostigmine
tandolapril	acetazolamide
tandolapril	methazolamide
tandolapril	dorzolamide
tandolapril	brinzolamide
phorbol ester	prostaglandin A
phorbol ester	prostaglandin B
phorbol ester	prostaglandin D
phorbol ester	prostaglandin E
phorbol ester	prostaglandin F
phorbol ester	latanaprost
phorbol ester	bimatoprost
phorbol ester	unoprostone
phorbol ester	travoprost
phorbol ester	betaxolol
phorbol ester	carteolol
phorbol ester	levobunolol
phorbol ester	metipranolol
phorbol ester	timolol
phorbol ester	levobetaxolol
phorbol ester	epinephrine
phorbol ester	dipivefrin
phorbol ester	pilocarpine
phorbol ester	pilocarpine hydrochloride
phorbol ester	carbachol
phorbol ester	demacarium
phorbol ester	echothiophate iodine
phorbol ester	physostigmine
phorbol ester	acetazolamide
phorbol ester	methazolamide
phorbol ester	dorzolamide
phorbol ester	brinzolamide
phorbol 12, 13-dibutyrate	prostaglandin A
phorbol 12, 13-dibutyrate	prostaglandin B
phorbol 12, 13-dibutyrate	prostaglandin D
phorbol 12, 13-dibutyrate	prostaglandin E
phorbol 12, 13-dibutyrate	prostaglandin F
phorbol 12, 13-dibutyrate	latanaprost
phorbol 12, 13-dibutyrate	bimatoprost
phorbol 12, 13-dibutyrate	unoprostone
phorbol 12, 13-dibutyrate	travoprost
phorbol 12, 13-dibutyrate	betaxolol
phorbol 12, 13-dibutyrate	carteolol
phorbol 12, 13-dibutyrate	levobunolol
phorbol 12, 13-dibutyrate	metipranolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
phorbol 12, 13-dibutyrate	timolol
phorbol 12, 13-dibutyrate	levobetaxolol
phorbol 12, 13-dibutyrate	epinephrine
phorbol 12, 13-dibutyrate	dipivefrin
phorbol 12, 13-dibutyrate	pilocarpine
phorbol 12, 13-dibutyrate	pilocarpine hydrochloride
phorbol 12, 13-dibutyrate	carbachol
phorbol 12, 13-dibutyrate	demacarium
phorbol 12, 13-dibutyrate	echothiophate iodine
phorbol 12, 13-dibutyrate	physostigmine
phorbol 12, 13-dibutyrate	acetazolamide
phorbol 12, 13-dibutyrate	methazolamide
phorbol 12, 13-dibutyrate	dorzolamide
phorbol 12, 13-dibutyrate	brinzolamide
phorbol 12-myristate-12-acetate	prostaglandin A
phorbol 12-myristate-12-acetate	prostaglandin B
phorbol 12-myristate-12-acetate	prostaglandin D
phorbol 12-myristate-12-acetate	prostaglandin E
phorbol 12-myristate-12-acetate	prostaglandin F
phorbol 12-myristate-12-acetate	latanaprost
phorbol 12-myristate-12-acetate	bimatoprost
phorbol 12-myristate-12-acetate	unoprostone
phorbol 12-myristate-12-acetate	travoprost
phorbol 12-myristate-12-acetate	betaxolol
phorbol 12-myristate-12-acetate	carteolol
phorbol 12-myristate-12-acetate	levobunolol
phorbol 12-myristate-12-acetate	metipranolol
phorbol 12-myristate-12-acetate	timolol
phorbol 12-myristate-12-acetate	levobetaxolol
phorbol 12-myristate-12-acetate	epinephrine
phorbol 12-myristate-12-acetate	dipivefrin
phorbol 12-myristate-12-acetate	pilocarpine
phorbol 12-myristate-12-acetate	pilocarpine hydrochloride
phorbol 12-myristate-12-acetate	carbachol
phorbol 12-myristate-12-acetate	demacarium
phorbol 12-myristate-12-acetate	echothiophate iodine
phorbol 12-myristate-12-acetate	physostigmine
phorbol 12-myristate-12-acetate	acetazolamide
phorbol 12-myristate-12-acetate	methazolamide
phorbol 12-myristate-12-acetate	dorzolamide
phorbol 12-myristate-12-acetate	brinzolamide
phorbol 12-O-tetradecanoylphorbol-13-acetate	prostaglandin A
phorbol 12-O-tetradecanoylphorbol-13-acetate	prostaglandin B

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
phorbol 12-O-tetradecanoylphorbol-13-acetate	prostaglandin D
phorbol 12-O-tetradecanoylphorbol-13-acetate	prostaglandin E
phorbol 12-O-tetradecanoylphorbol-13-acetate	prostaglandin F
phorbol 12-O-tetradecanoylphorbol-13-acetate	latanaprost
phorbol 12-O-tetradecanoylphorbol-13-acetate	bimatoprost
phorbol 12-O-tetradecanoylphorbol-13-acetate	unoprostone
phorbol 12-O-tetradecanoylphorbol-13-acetate	travoprost
phorbol 12-O-tetradecanoylphorbol-13-acetate	betaxolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	carteolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	levobunolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	metipranolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	timolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	levobetaxolol
phorbol 12-O-tetradecanoylphorbol-13-acetate	epinephrine
phorbol 12-O-tetradecanoylphorbol-13-acetate	dipivefrin
phorbol 12-O-tetradecanoylphorbol-13-acetate	pilocarpine
phorbol 12-O-tetradecanoylphorbol-13-acetate	pilocarpine hydrochloride
phorbol 12-O-tetradecanoylphorbol-13-acetate	carbachol
phorbol 12-O-tetradecanoylphorbol-13-acetate	demacarium
phorbol 12-O-tetradecanoylphorbol-13-acetate	echothiophate iodine
phorbol 12-O-tetradecanoylphorbol-13-acetate	physostigmine
phorbol 12-O-tetradecanoylphorbol-13-acetate	acetazolamide
phorbol 12-O-tetradecanoylphorbol-13-acetate	methazolamide

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
phorbol 12-O-tetradecanoylphorbol-13-acetate	dorzolamide
phorbol 12-O-tetradecanoylphorbol-13-acetate	brinzolamide
phorbol 12, 13-didecanoate	prostaglandin A
phorbol 12, 13-didecanoate	prostaglandin B
phorbol 12, 13-didecanoate	prostaglandin D
phorbol 12, 13-didecanoate	prostaglandin E
phorbol 12, 13-didecanoate	prostaglandin F
phorbol 12, 13-didecanoate	latanaprost
phorbol 12, 13-didecanoate	bimatoprost
phorbol 12, 13-didecanoate	unoprostone
phorbol 12, 13-didecanoate	travoprost
phorbol 12, 13-didecanoate	betaxolol
phorbol 12, 13-didecanoate	carteolol
phorbol 12, 13-didecanoate	levobunolol
phorbol 12, 13-didecanoate	metipranolol
phorbol 12, 13-didecanoate	timolol
phorbol 12, 13-didecanoate	levobetaxolol
phorbol 12, 13-didecanoate	epinephrine
phorbol 12, 13-didecanoate	dipivefrin
phorbol 12, 13-didecanoate	pilocarpine
phorbol 12, 13-didecanoate	pilocarpine hydrochloride
phorbol 12, 13-didecanoate	carbachol
phorbol 12, 13-didecanoate	demacarium
phorbol 12, 13-didecanoate	echothiophate iodine
phorbol 12, 13-didecanoate	physostigmine
phorbol 12, 13-didecanoate	acetazolamide
phorbol 12, 13-didecanoate	methazolamide
phorbol 12, 13-didecanoate	dorzolamide
phorbol 12, 13-didecanoate	brinzolamide
tetradecanoylphorbol acetate	prostaglandin A
tetradecanoylphorbol acetate	prostaglandin B
tetradecanoylphorbol acetate	prostaglandin D
tetradecanoylphorbol acetate	prostaglandin E
tetradecanoylphorbol acetate	prostaglandin F
tetradecanoylphorbol acetate	latanaprost
tetradecanoylphorbol acetate	bimatoprost
tetradecanoylphorbol acetate	unoprostone
tetradecanoylphorbol acetate	travoprost
tetradecanoylphorbol acetate	betaxolol
tetradecanoylphorbol acetate	carteolol
tetradecanoylphorbol acetate	levobunolol
tetradecanoylphorbol acetate	metipranolol
tetradecanoylphorbol acetate	timolol
tetradecanoylphorbol acetate	levobetaxolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
tetradecanoylphorbol acetate	epinephrine
tetradecanoylphorbol acetate	dipivefrin
tetradecanoylphorbol acetate	pilocarpine
tetradecanoylphorbol acetate	pilocarpine hydrochloride
tetradecanoylphorbol acetate	carbachol
tetradecanoylphorbol acetate	demacarium
tetradecanoylphorbol acetate	echothiophate iodine
tetradecanoylphorbol acetate	physostigmine
tetradecanoylphorbol acetate	acetazolamide
tetradecanoylphorbol acetate	methazolamide
tetradecanoylphorbol acetate	dorzolamide
tetradecanoylphorbol acetate	brinzolamide
ionomycin	prostaglandin A
ionomycin	prostaglandin B
ionomycin	prostaglandin D
ionomycin	prostaglandin E
ionomycin	prostaglandin F
ionomycin	latanaprost
ionomycin	bimatoprost
ionomycin	unoprostone
ionomycin	travoprost
ionomycin	betaxolol
ionomycin	carteolol
ionomycin	levobunolol
ionomycin	metipranolol
ionomycin	timolol
ionomycin	levobetaxolol
ionomycin	epinephrine
ionomycin	dipivefrin
ionomycin	pilocarpine
ionomycin	pilocarpine hydrochloride
ionomycin	carbachol
ionomycin	demacarium
ionomycin	echothiophate iodine
ionomycin	physostigmine
ionomycin	acetazolamide
ionomycin	methazolamide
ionomycin	dorzolamide
ionomycin	brinzolamide
vasopressin	prostaglandin A
vasopressin	prostaglandin B
vasopressin	prostaglandin D
vasopressin	prostaglandin E
vasopressin	prostaglandin F
vasopressin	latanaprost
vasopressin	bimatoprost

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
vasopressin	unoprostone
vasopressin	travoprost
vasopressin	betaxolol
vasopressin	carteolol
vasopressin	levobunolol
vasopressin	metipranolol
vasopressin	timolol
vasopressin	levobetaxolol
vasopressin	epinephrine
vasopressin	dipivefrin
vasopressin	pilocarpine
vasopressin	pilocarpine hydrochloride
vasopressin	carbachol
vasopressin	demacarium
vasopressin	echothiophate iodine
vasopressin	physostigmine
vasopressin	acetazolamide
vasopressin	methazolamide
vasopressin	dorzolamide
vasopressin	brinzolamide
arginine vasopressin	prostaglandin A
arginine vasopressin	prostaglandin B
arginine vasopressin	prostaglandin D
arginine vasopressin	prostaglandin E
arginine vasopressin	prostaglandin F
arginine vasopressin	latanaprost
arginine vasopressin	bimatoprost
arginine vasopressin	unoprostone
arginine vasopressin	travoprost
arginine vasopressin	betaxolol
arginine vasopressin	carteolol
arginine vasopressin	levobunolol
arginine vasopressin	metipranolol
arginine vasopressin	timolol
arginine vasopressin	levobetaxolol
arginine vasopressin	epinephrine
arginine vasopressin	dipivefrin
arginine vasopressin	pilocarpine
arginine vasopressin	pilocarpine hydrochloride
arginine vasopressin	carbachol
arginine vasopressin	demacarium
arginine vasopressin	echothiophate iodine
arginine vasopressin	physostigmine
arginine vasopressin	acetazolamide
arginine vasopressin	methazolamide
arginine vasopressin	dorzolamide

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
arginine vasopressin	brinzolamide
atrial natriuretic peptide	prostaglandin A
atrial natriuretic peptide	prostaglandin B
atrial natriuretic peptide	prostaglandin D
atrial natriuretic peptide	prostaglandin E
atrial natriuretic peptide	prostaglandin F
atrial natriuretic peptide	latanaprost
atrial natriuretic peptide	bimatoprost
atrial natriuretic peptide	unoprostone
atrial natriuretic peptide	travoprost
atrial natriuretic peptide	betaxolol
atrial natriuretic peptide	carteolol
atrial natriuretic peptide	levobunolol
atrial natriuretic peptide	metipranolol
atrial natriuretic peptide	timolol
atrial natriuretic peptide	levobetaxolol
atrial natriuretic peptide	epinephrine
atrial natriuretic peptide	dipivefrin
atrial natriuretic peptide	pilocarpine
atrial natriuretic peptide	pilocarpine hydrochloride
atrial natriuretic peptide	carbachol
atrial natriuretic peptide	demacarium
atrial natriuretic peptide	echothiophate iodine
atrial natriuretic peptide	physostigmine
atrial natriuretic peptide	acetazolamide
atrial natriuretic peptide	methazolamide
atrial natriuretic peptide	dorzolamide
atrial natriuretic peptide	brinzolamide
brain natriuretic peptide	prostaglandin A
brain natriuretic peptide	prostaglandin B
brain natriuretic peptide	prostaglandin D
brain natriuretic peptide	prostaglandin E
brain natriuretic peptide	prostaglandin F
brain natriuretic peptide	latanaprost
brain natriuretic peptide	bimatoprost
brain natriuretic peptide	unoprostone
brain natriuretic peptide	travoprost
brain natriuretic peptide	betaxolol
brain natriuretic peptide	carteolol
brain natriuretic peptide	levobunolol
brain natriuretic peptide	metipranolol
brain natriuretic peptide	timolol
brain natriuretic peptide	levobetaxolol
brain natriuretic peptide	epinephrine
brain natriuretic peptide	dipivefrin
brain natriuretic peptide	pilocarpine

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
brain natriuretic peptide	pilocarpine hydrochloride
brain natriuretic peptide	carbachol
brain natriuretic peptide	demacarium
brain natriuretic peptide	echothiophate iodine
brain natriuretic peptide	physostigmine
brain natriuretic peptide	acetazolamide
brain natriuretic peptide	methazolamide
brain natriuretic peptide	dorzolamide
brain natriuretic peptide	brinzolamide
tetraethyl ammonium	prostaglandin A
tetraethyl ammonium	prostaglandin B
tetraethyl ammonium	prostaglandin D
tetraethyl ammonium	prostaglandin E
tetraethyl ammonium	prostaglandin F
tetraethyl ammonium	latanaprost
tetraethyl ammonium	bimatoprost
tetraethyl ammonium	unoprostone
tetraethyl ammonium	travoprost
tetraethyl ammonium	betaxolol
tetraethyl ammonium	carteolol
tetraethyl ammonium	levobunolol
tetraethyl ammonium	metipranolol
tetraethyl ammonium	timolol
tetraethyl ammonium	levobetaxolol
tetraethyl ammonium	epinephrine
tetraethyl ammonium	dipivefrin
tetraethyl ammonium	pilocarpine
tetraethyl ammonium	pilocarpine hydrochloride
tetraethyl ammonium	carbachol
tetraethyl ammonium	demacarium
tetraethyl ammonium	echothiophate iodine
tetraethyl ammonium	physostigmine
tetraethyl ammonium	acetazolamide
tetraethyl ammonium	methazolamide
tetraethyl ammonium	dorzolamide
tetraethyl ammonium	brinzolamide
colchicine	prostaglandin A
colchicine	prostaglandin B
colchicine	prostaglandin D
colchicine	prostaglandin E
colchicine	prostaglandin F
colchicine	latanaprost
colchicine	bimatoprost
colchicine	unoprostone
colchicine	travoprost
colchicine	betaxolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
colchicine	carteolol
colchicine	levobunolol
colchicine	metipranolol
colchicine	timolol
colchicine	levobetaxolol
colchicine	epinephrine
colchicine	dipivefrin
colchicine	pilocarpine
colchicine	pilocarpine hydrochloride
colchicine	carbachol
colchicine	demacarium
colchicine	echothiophate iodine
colchicine	physostigmine
colchicine	acetazolamide
colchicine	methazolamide
colchicine	dorzolamide
colchicine	brinzolamide
vinca alkaloid	prostaglandin A
vinca alkaloid	prostaglandin B
vinca alkaloid	prostaglandin D
vinca alkaloid	prostaglandin E
vinca alkaloid	prostaglandin F
vinca alkaloid	latanaprost
vinca alkaloid	bimatoprost
vinca alkaloid	unoprostone
vinca alkaloid	travoprost
vinca alkaloid	betaxolol
vinca alkaloid	carteolol
vinca alkaloid	levobunolol
vinca alkaloid	metipranolol
vinca alkaloid	timolol
vinca alkaloid	levobetaxolol
vinca alkaloid	epinephrine
vinca alkaloid	dipivefrin
vinca alkaloid	pilocarpine
vinca alkaloid	pilocarpine hydrochloride
vinca alkaloid	carbachol
vinca alkaloid	demacarium
vinca alkaloid	echothiophate iodine
vinca alkaloid	physostigmine
vinca alkaloid	acetazolamide
vinca alkaloid	methazolamide
vinca alkaloid	dorzolamide
vinca alkaloid	brinzolamide
rhizoxin	prostaglandin A
rhizoxin	prostaglandin B

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
rhizoxin	prostaglandin D
rhizoxin	prostaglandin E
rhizoxin	prostaglandin F
rhizoxin	latanaprost
rhizoxin	bimatoprost
rhizoxin	unoprostone
rhizoxin	travoprost
rhizoxin	betaxolol
rhizoxin	carteolol
rhizoxin	levobunolol
rhizoxin	metipranolol
rhizoxin	timolol
rhizoxin	levobetaxolol
rhizoxin	epinephrine
rhizoxin	dipivefrin
rhizoxin	pilocarpine
rhizoxin	pilocarpine hydrochloride
rhizoxin	carbachol
rhizoxin	demacarium
rhizoxin	echothiophate iodine
rhizoxin	physostigmine
rhizoxin	acetazolamide
rhizoxin	methazolamide
rhizoxin	dorzolamide
rhizoxin	brinzolamide
estramustine	prostaglandin A
estramustine	prostaglandin B
estramustine	prostaglandin D
estramustine	prostaglandin E
estramustine	prostaglandin F
estramustine	latanaprost
estramustine	bimatoprost
estramustine	unoprostone
estramustine	travoprost
estramustine	betaxolol
estramustine	carteolol
estramustine	levobunolol
estramustine	metipranolol
estramustine	timolol
estramustine	levobetaxolol
estramustine	epinephrine
estramustine	dipivefrin
estramustine	pilocarpine
estramustine	pilocarpine hydrochloride
estramustine	carbachol
estramustine	demacarium

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
estramustine	echothiophate iodine
estramustine	physostigmine
estramustine	acetazolamide
estramustine	methazolamide
estramustine	dorzolamide
estramustine	brinzolamide
nocodazole	prostaglandin A
nocodazole	prostaglandin B
nocodazole	prostaglandin D
nocodazole	prostaglandin E
nocodazole	prostaglandin F
nocodazole	latanaprost
nocodazole	bimatoprost
nocodazole	unoprostone
nocodazole	travoprost
nocodazole	betaxolol
nocodazole	carteolol
nocodazole	levobunolol
nocodazole	metipranolol
nocodazole	timolol
nocodazole	levobetaxolol
nocodazole	epinephrine
nocodazole	dipivefrin
nocodazole	pilocarpine
nocodazole	pilocarpine hydrochloride
nocodazole	carbachol
nocodazole	demacarium
nocodazole	echothiophate iodine
nocodazole	physostigmine
nocodazole	acetazolamide
nocodazole	methazolamide
nocodazole	dorzolamide
nocodazole	brinzolamide
erbuluzole	prostaglandin A
erbuluzole	prostaglandin B
erbuluzole	prostaglandin D
erbuluzole	prostaglandin E
erbuluzole	prostaglandin F
erbuluzole	latanaprost
erbuluzole	bimatoprost
erbuluzole	unoprostone
erbuluzole	travoprost
erbuluzole	betaxolol
erbuluzole	carteolol
erbuluzole	levobunolol
erbuluzole	metipranolol

TABLE 4	
Aquaporin Modulating Agent	**Aqueous Humor Modulating Agent**
erbuluzole	timolol
erbuluzole	levobetaxolol
erbuluzole	epinephrine
erbuluzole	dipivefrin
erbuluzole	pilocarpine
erbuluzole	pilocarpine hydrochloride
erbuluzole	carbachol
erbuluzole	demacarium
erbuluzole	echothiophate iodine
erbuluzole	physostigmine
erbuluzole	acetazolamide
erbuluzole	methazolamide
erbuluzole	dorzolamide
erbuluzole	brinzolamide
tubulozole	prostaglandin A
tubulozole	prostaglandin B
tubulozole	prostaglandin D
tubulozole	prostaglandin E
tubulozole	prostaglandin F
tubulozole	latanaprost
tubulozole	bimatoprost
tubulozole	unoprostone
tubulozole	travoprost
tubulozole	betaxolol
tubulozole	carteolol
tubulozole	levobunolol
tubulozole	metipranolol
tubulozole	timolol
tubulozole	levobetaxolol
tubulozole	epinephrine
tubulozole	dipivefrin
tubulozole	pilocarpine
tubulozole	pilocarpine hydrochloride
tubulozole	carbachol
tubulozole	demacarium
tubulozole	echothiophate iodine
tubulozole	physostigmine
tubulozole	acetazolamide
tubulozole	methazolamide
tubulozole	dorzolamide
tubulozole	brinzolamide

Diagnosis of an Elevated IOP or an Ophthalmic Disorder

[0083] One aspect of the invention encompasses diagnosing a subject in need of treatment for lowering intraocular pressure or in need of treatment for an ophthalmic disorder. A number of suitable methods for diagnosing a subject in need of treatment for lowering intraocular pressure or in need of treatment for an ophthalmic disorder may be used in the practice of the invention. While the type of test employed for diagnosis is dependent upon the subject's physical symptoms, a routine eye examine is generally performed in most embodiments. A routine eye exam usually includes measuring a subject's eye pressure with any of a number of reliable instruments known in the art, such as devices that record measurements based upon a puff of air into a subject's eye. Typically, the eye exam will also include an examination of the meshwork as well. In one embodiment, the pupils are dilated so as to allow examination of the meshwork and optic nerve. The eye exam may also consist of an examination of the optic disc, such as by using three-dimensional photography. In addition, a formal examination of the peripheral field of vision is also typically carried out with a computerized visual field machine.

Indications to be Treated

[0084] The composition comprising a therapeutically effective amount of an AQP modulating agent and a therapeutically effective amount of an aqueous humor modulating agent may be employed to treat any condition resulting from elevated IOP, low IOP or aberrant ocular water transport in a subject.

[0085] In some aspects, the invention provides a method for lowering IOP in a subject. The composition may be utilized to treat any ophthalmic disorder in a subject mediated by elevated IOP. Elevated IOP is typically a level of IOP that is harmful to the optic nerve in a particular subject and can readily be determined by a skilled artisan. The IOP may be within the normal range, particularly in patients with normal pressure glaucoma. By way of example, glaucoma is characterized by a progressive neuropathy caused in part by deleterious effects resulting from increased IOP on the optic nerve. In normal individuals, IOPs range from 12 to 20 mm Hg., averaging approximately 16 mm Hg. At higher values, for instance over 22 mm Hg, there is a risk that the eye may be affected, and if left untreated, result in the formation of glaucoma.

[0086] In one embodiment, the composition may be administered to a subject where elevated IOP or aberrant ocular water transport in a subject is a causative factor in the formation of any type of glaucoma. Several different types of glaucomas exist, each having different pathophysiologies and risk factors may be treated by administration of the composition of the invention. In terms of classification, glaucomas may first be deemed to be either "primary" or "secondary." Primary glaucomas, result directly from anatomical and/or physiological disturbances in the flow of aqueous humor, which in turn causes IOP to rise. Secondary glaucomas occur as a sequel to ocular injury (e.g., trauma inflicted to the eye) or preexisting disease (e.g., an intraocular tumor or an enlarged cataract). Though the various secondary glaucomas have different etiologies, they are similar to the primary glaucomas in that they all produce visual loss through optic neuropathy.

[0087] The composition may be advantageously administered to a subject with any form of primary glaucoma. In one alternative of this embodiment, the primary glaucoma is open-angle glaucoma (also known as chronic or simple glaucoma). Open angle glaucoma is characterized by abnormally high resistance to fluid drainage from the eye. In another alternative of this embodiment, the primary glaucoma is angle-closure glaucoma (also known as closed-angle or narrow-angle glaucoma). Angle-closure glaucoma entails closure or blockage of the anterior chamber angle by another ocular structure (usually the iris), thereby restricting outflow of aqueous humor. In still another alternative of this embodiment, the primary glaucoma is congenital glaucoma (also known as infantile glaucoma).

[0088] In another embodiment, the composition may be advantageously administered to a subject with any form of secondary glaucoma. By way of example, the secondary glaucoma may be secondary open angle glaucoma or secondary angle closure glaucoma.

[0089] In still a further embodiment, the composition is administered to subjects that have ocular hypertension, but have not yet developed glaucoma. In this embodiment, typically the subject will have an IOP greater than about 20 mm Hg, more typically greater than 21 mm Hg and even more typically, greater than about 22 mm Hg.

[0090] In yet a further embodiment, the composition may be administered to a subject having a high risk for the development of glaucoma. In addition to subjects having elevated IOP, certain groups of subjects are at risk for developing

glaucoma. These groups typically include subjects with a family history of glaucoma, persons of African descent over age 40, everyone over age 60, and diabetics. In one alternative of this embodiment, the subject also has an elevated IOP.

[0091] In another embodiment, the composition may be administered to a subject taking a particular drug known to increase the incidence of glaucoma. By way of example, the corticosteroids (e.g., prednisone, dexamethasone, and hydrocortisone) are known to induce glaucoma following both ophthalmic and systemic administration systemic administration, by increasing resistance to aqueous humor outflow through the trabecular meshwork via a mechanism somehow genetically linked to primary open angle glaucoma. In particular, dexamethasone has been associated with the most pronounced increase in intraocular pressure, and ophthalmic administration generally leads to greater increases than systemic administration.

[0092] In another aspect, the composition may be administered to a subject having an ophthalmic disorder mediated by aberrant ocular water transport. By way of example, the ophthalmic disorder may be idiopathic macular edema, corneal edema, diabetic macular edema, post-cataract macular edema, central serous retinopathy or any venous occlusive disorder of the retina.

EXAMPLES

[0093] In the examples below, a combination therapy contains an aqueous humor modulating agent and an aquaporin modulating agent. The efficacy of such combination therapy can be evaluated in comparison to a control treatment such as a placebo treatment, administration of an aquaporin modulating agent only, or administration of an aqueous humor modulating agent only. By way of example, a combination therapy may contain a vasoactive peptide and a prostanglandin or prostaglandin analog, an angiotensin converting enzyme inhibitor and a cholinergic agonist, a protein kinase C activator and a beta adrenergic antagonist, a protein A inhibitor and carbonic anhydrase inhibitor, or a vasoactive peptide and an adrenergic agonist. It should be noted that these are only several examples, and that any of the aquaporin modulating agents and aqueous humor modulating agents detailed in the present invention, including the combinations set forth in Tables 3 or 4 may be tested as a combination therapy. The dosages of an aqueous humor modulating agent and an aquaporin modulating agent in a particular therapeutic combination may be

readily determined by a skilled artisan conducting the study. The length of the study treatment will vary on a particular study and can also be determined by one of ordinary skill in the art. By way of example, the combination therapy may be administered for 12 weeks. The composition can be administered by any route as described herein, but is preferably administered as an ocular formulation directly to the eye of the subject being tested.

IOP Animal Study

[0094] The laboratory animal study can generally be performed as described in Savinova *et al.*, *BMC Genetics* 2:12, Aug. 9, 2001.

Animal husbandry

[0095] All experiments are performed in compliance with the ARVO statement for use of animals in ophthalmic and vision research. Briefly, mice are housed in cages containing white pine bedding and covered with polyester filters. For most experiments, the mice are fed NIH31 (6 % fat) chow *ad libitum*, and their water is acidified to pH 2.8 to 3.2. The mice are housed based on the experimental group and the cages are changed one time per week. If any cage appears soiled between scheduled changes, the mice are placed in a clean cage. The environment is kept at 21°C with a 14 hour light: 10 hour dark cycle. The colony is monitored for specific pathogens routinely.

[0096] Mice chosen for this study can be of C57BL/6J (Bl/6) strain; however, other strains can also be used. Since glaucoma, which is associated with high intraocular pressure generally occurs in older individuals, mice used herein are older, between about 12 months and 24 months of age. It should be noted that the same experiment can be performed with younger animals, if desired. Control mice are selected from the same strain and same age group as the experimental mice (receiving combination therapy). By way of example, if the experimental group comprises 10 Bl/6 mice, 3 Bl/6 mice can be used as a control.

[0097] Mice that have elevated intraocular pressure can also be used in this study. For example, mice that are heterozygous for bone morphogenetic protein 4 ($Bmp4^{+}/.$ mice) have anterior segment abnormalities including malformed, absent or blocked trabecular meshwork and Schlemm's canal drainage structures. Mice with severe drainage structure abnormalities over 80% or more of their angle's extent

have elevated IOP. The penetrance and severity of abnormalities is strongly influenced by genetic background, being most severe on the Bl/6 background. On the Bl/6 background, there is a persistence of hyaloid vasculature, diminished numbers of inner retinal cells, and absence of the optic nerve. See, e.g., Chang *et al.*, BMC Genetics, 2:18, Nov. 6, 2001. Accordingly, an experimental group can consist of $Bmp4^{+}/_{-}$ mice receiving combination therapy, whereas the control group consists of $Bmp4^{+}/_{-}$ mice receiving a placebo treatment. The placebo treatment can be readily determined by a skilled artisan; for example, if the combination therapy is administered intravenously or intraperitoneally, the vehicle used for such administration can be used as a placebo.

Combination Treatment

[0098] Mice in the experimental group are administered the combination therapy as described above by any of the acceptable routes, e.g., intraperitoneal or intravenous. The duration and frequency of the treatment can readily be determined by a skilled artisan. By way of example, the combination therapy can be administered once a day for a period of 2 weeks. The amount of the therapy to be administered can also be readily determined by one skilled in the art. Control mice are treated according to the same protocol, except that they are administered a placebo rather than a combination therapy. Following the treatment, eyes of both the experimental and control mice are examined to determine the effect of the treatment. The result can be evaluated by determining intraocular pressure, and e.g., by performing immunohistochemistry on the eyes. For example, histochemistry (performed as described below) can be used to determine if the iridocorneal angle and aqueous humor drainage structures are open to the anterior chamber and have normal morphology.

Intraocular pressure (IOP)

[0099] Intraocular pressure is measured as described, for example, in John SWM, Hagaman JR, MacTaggart TE, Peng L, Smithes O: Intraocular pressure in inbred mouse strains, *Invest. Ophthalmol. Vis. Sci.* 1997, 38:249-253. The mice are typically acclimatized to the procedure room for at least 2 weeks prior to measurement, but sometimes between 1 and 2 weeks.

[00100] All dark period measurements are made between 1 and 3 hours after the lights are turned off. The room is equipped with dim red lights and mice are protected from all light exposure during set up. Each mouse is briefly exposed to the red light when the anesthetic agents are administered. When adequate anesthesia is achieved (after 3 to 4 minutes), the mouse is placed on the measurement platform and the white light of the microscope is turned on (for approximately 1 and a half minutes) to allow ocular cannulation. The white light is used at very low intensity and is dim to minimize, if not eliminate possibility that this brief exposure alters the IOP. All other mice are protected from light exposure throughout the time an individual mouse is analyzed.

Clinical examinations

[00101] Anterior chambers are examined with a slit lamp and photographs are taken using a 40X objective lens. An indirect ophthalmoscope and a 60 or 90 diopter lens is used to visualize the retinas and optic nerves. For this analysis, pupils are dilated with a drop of 1% cyclopentolate.

Histological analysis

[00102] Eyes from at least several mice from the experimental and control group are fixed (4% paraformaldehyde or Fekete's acid-alcohol-formalin fixative) processed, paraffin embedded and sectioned as previously reported[1], except that the paraformaldehyde is buffered with 0.1 M phosphate buffer. A number of the eyes are processed for plastic embedding (Historesin, Leica, Heidelberg, Germany), and sectioned as previously reported[2]. Saggital sections including the pupil and optic nerve are collected and analyzed as they contain most ocular structures.

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[1] Chang et al., Nat. Genet. 1999, 21:405-409 and Smith RS, Nishina PM, Ikeda S, Jewett P, Zabaleta A, John SWM: Interpretation of Ocular Pathology in Genetically-Engineered and Spontaneous Mutant Mice. In: Pathology of Genetically Engineered Mice Edited by Ward J, Sundberg J. pp. 217–231. Iowa: University of Iowa Press; 2000, 217-231

[2] John et al., *Invest. Ophthalmol. Vis. Sci.* 1998, 39:951-962 and Smith RS, Nishina PM, Ikeda S, Jewett P, Zabaleta A, John SWM: Interpretation of Ocular Pathology in Genetically-Engineered and Spontaneous Mutant Mice. In: Pathology of Genetically Engineered Mice Edited by Ward J, Sundberg J. pp. 217–231. Iowa: University of Iowa Press; 2000, 217-231

Results

[00103] Older BI/6 mice can be used to determine if the combination therapy provides a prophylactic or therapeutic (if the mice have a high IOP) benefit. The benefit(s) can be evaluated by determining IOP levels prior and post treatment. Furthermore, the histology can be used to evaluate the presence or absence of pathological ocular features before and after the treatment.

[00104] When $Bmp4^{+/-}$ mice are used, it is expected that the combination therapy will result in a decrease in IOP in these mice following the treatment regimen. Eye histochemistry as described above can also be used to evaluate whether the treatment results in any improvement of drainage structure abnormalities.

[00105] It should be noted that all of the above-mentioned procedures can be modified for a particular study, depending on factors such as a drug combination used, length of the study, subjects that are selected, etc. Such modifications can be designed by a skilled artisan without undue experimentation.

WHAT IS CLAIMED IS:

1. A method of lowering intraocular pressure, the method comprising:

(a) diagnosing a subject for a condition mediated by elevated intraocular pressure; and

(b) administering to the subject a combination comprising an aquaporin modulating agent and an aqueous humor modulating agent, wherein the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin.

2. The method of claim 1 wherein the aquaporin modulating agent is selected from the group consisting of an angiotensin converting enzyme inhibitor, a protein kinase C activator, a protein kinase A inhibitor, a vasoactive peptide, and a vinca alkaloid.

3. The method of claim 2 wherein the aqueous humor modulating agent is selected from the group consisting of a prostaglandin, a prostaglandin analog, a beta adrenergic antagonist, an adrenergic agonist, a cholinergic agonist and a carbonic anhydrase inhibitor.

4. The method of claim 1 wherein the aquaporin modulating agent is selected from the group consisting of enalapril, benazepril, captopril, fosinopril, lisinopril, moexipril, quinapril, ramipril, trandolapril, phorbol 12, 13 dibutyrate, phorbol 12-myristate-12-acetate, phorbol 12-O-tetradecanoylphorbol 13-acetate, phorbol 12, 13 didecanoate, tetradecanoylphorbol acetate, ionomycin, arginine vasopressin, atrial natriuretic peptide, brain natriuretic peptide, tetraethylammonium, colchicine, rhizoxin, estramustine, nocodazole, erbuluzole, and tubulozole.

5. The method of claim 4 wherein the aqueous humor modulating agent is selected from the group consisting of prostaglandin A, prostaglandin B, prostaglandin D, prostaglandin E, prostaglandin F, latanaprost, bimatoprost, unoprostone, travoprost, betaxolol, carteolol, levobunolol, metipranolol, timolol, levobetaxolol, epinephrine, dipivefrin, pilocarpine, pilocarpine hydrochloride, carbachol, demacarium, echothiophate idodine, physostigmine, acetazolamide,

methazolamide, dorzolamide hydrochloride ophthalmic solution, dorzolamide hydrochloride-timolol maleate ophthalmic solution, brinzolamide hydrochloride, dorzolamide, and brinzolamide.

6. A method of treating an ophthalmic disorder in a subject, the method comprising:

(a) diagnosing a subject in need of treatment for an ophthalmic disorder; and

(b) administering to the subject a combination comprising an aquaporin modulating agent and an aqueous humor modulating agent, wherein the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin.

7. The method of claim 6 wherein the aquaporin modulating agent is selected from the group consisting of an angiotensin converting enzyme inhibitor, a protein kinase C activator, a protein kinase A inhibitor, a vasoactive peptide, and a vinca alkaloid.

8. The method of claim 7 wherein the aqueous humor modulating agent is selected from the group consisting of a prostaglandin, a prostaglandin analog, a beta adrenergic antagonist, an adrenergic agonist, a cholinergic agonist and a carbonic anhydrase inhibitor.

9. The method of claim 6 wherein the aquaporin modulating agent is selected from the group consisting of enalapril, benazepril, captopril, fosinopril, lisinopril, moexipril, quinapril, ramipril, trandolapril, phorbol 12, 13 dibutyrate, phorbol 12-myristate-12-acetate, phorbol 12-O-tetradecanoylphorbol 13-acetate, phorbol 12, 13 didecanoate, tetradecanoylphorbol acetate, ionomycin, arginine vasopressin, atrial natriuretic peptide, brain natriuretic peptide, tetraethylammonium, colchicine, rhizoxin, estramustine, nocodazole, erbuluzole, and tubulozole.

10. The method of claim 9 wherein the aqueous humor modulating agent is selected from the group consisting of prostaglandin A, prostaglandin B, prostaglandin D, prostaglandin E, prostaglandin F, latanaprost, bimatoprost,

unoprostone, travoprost, betaxolol, carteolol, levobunolol, metipranolol, timolol, levobetaxolol, epinephrine, dipivefrin, pilocarpine, pilocarpine hydrochloride, carbachol, demacarium, echothiophate idodine, physostigmine, acetazolamide, methazolamide, dorzolamide hydrochloride ophthalmic solution, dorzolamide hydrochloride-timolol maleate ophthalmic solution, brinzolamide hydrochloride, dorzolamide, and brinzolamide.

11. A method of treating glaucoma in a subject, the method comprising:

(a) diagnosing a subject in need of treatment for glaucoma; and

(b) administering to the subject a combination comprising an aquaporin modulating agent and an aqueous humor modulating agent, wherein the aqueous humor modulating agent lowers intraocular pressure by a pathway other than the modulation of aquaporin.

12. The method of claim 11 wherein the aquaporin modulating agent is selected from the group consisting of an angiotensin converting enzyme inhibitor, a protein kinase C activator, a protein kinase A inhibitor, a vasoactive peptide, and a vinca alkaloid.

13. The method of claim 12 wherein the aqueous humor modulating agent is selected from the group consisting of a prostaglandin, a prostaglandin analog, a beta adrenergic antagonist, an adrenergic agonist, a cholinergic agonist and a carbonic anhydrase inhibitor.

14. The method of claim 11 wherein the aquaporin modulating agent is selected from the group consisting of enalapril, benazepril, captopril, fosinopril, lisinopril, moexipril, quinapril, ramipril, trandolapril, phorbol 12, 13 dibutyrate, phorbol 12-myristate-12-acetate, phorbol 12-O-tetradecanoylphorbol 13-acetate, phorbol 12, 13 didecanoate, tetradecanoylphorbol acetate, ionomycin, arginine vasopressin, atrial natriuretic peptide, brain natriuretic peptide, tetraethylammonium, colchicine, rhizoxin, estramustine, nocodazole, erbuluzole, and tubulozole.

15. The method of claim 14 wherein the aqueous humor modulating agent is selected from the group consisting of prostaglandin A, prostaglandin B,

prostaglandin D, prostaglandin E, prostaglandin F, latanaprost, bimatoprost, unoprostone, travoprost, betaxolol, carteolol, levobunolol, metipranolol, timolol, levobetaxolol, epinephrine, dipivefrin, pilocarpine, pilocarpine hydrochloride, carbachol, demacarium, echothiophate idodine, physostigmine, acetazolamide, methazolamide, dorzolamide hydrochloride ophthalmic solution, dorzolamide hydrochloride-timolol maleate ophthalmic solution, brinzolamide hydrochloride, dorzolamide, and brinzolamide.